12-2012

Sorption and Enantiomerization of Current Use Chiral Pesticides

April Hall
Clemson University, april.hall@nutramfg.com

SORPTION AND ENANTIOMERIZATION OF CURRENT USE CHIRAL PESTICIDES

A Dissertation
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Doctors of Philosophy
Environmental Engineering and Earth Sciences

---

by
April A. Hall
December 2012

Accepted by:
Dr. Cindy M. Lee, Committee Chair
Dr. Alan W. Elzerman
Dr. David L. Freedman
Dr. Arthur W. Garrison

ABSTRACT

Chiral pesticides are prevalent in the environment today and are known to react selectively with chiral environmental components such as microbes, enzymes, and other naturally occurring chiral materials. In addition, chiral sorption is a process that has been occasionally investigated in the study of homochirality (the exclusive presence of one enantiomer in living organisms), but almost overlooked in environmental science. For chiral sorption to occur, the sorbent and sorbate must be chiral entities. In the environment, there are abundant natural surfaces that are chiral, including clay minerals and organic matter present in soil, sediment and aqueous solution. A knowledge of isomerization, including the special case of enantiomerization, of chiral pesticides is also crucial in understanding the fate of these chiral pollutants, especially for pesticides marketed as a “chiral switch":, i.e. formulations containing only or mostly the active enantiomer. The goal of this research was to provide more insight into sorption and enantiomerization of chiral pesticides in the presence of mineral surfaces.

Sorption experiments with malathion and metalaxyl and selected minerals, along with their individual enantiomers, were conducted and results analyzed by achiral and chiral high performance liquid chromatography (HPLC). Investigation with racemic malathion resulted in significant sorption to all sorbents, with the exception of the negligible sorption to kaolinite. Sorption was fit to the Freundlich model, with $n_{2hr} > 1$ for bentonite, calcite, and montmorillonite while the biotic solids diatomaceous earth

and seashells had $n_{2hr} < 1$. $K_{F,2hr}$ values indicated decreasing sorption capacity in the order montmorillonite, bentonite, calcite, seashells, and diatomaceous earth. Chiral analysis indicated nonenantioselective sorption for malathion, which exhibited racemic enantiomeric fractions (EFs) for all sorbents.

Sorption of the individual enantiomers of malathion, *R*-(*+*)- and *S*-(-)-malathion, resulted in enantiomerization to racemic EFs after contact with the mineral sorbents. Adding malathion enantiomers to minerals and analyzing the aqueous phase after 15 min of centrifugation resulted in enantiomerization to racemic EF values for *R*-(*+*)-malathion, but only partial enantiomerization of *S*-(-)-malathion. Malathion enantiomers also decreased or increased in EF for controls containing either only water or β-cyclodextrin, but enantiomerization to the racemate was not complete after two hours. It was hypothesized that malathion is undergoing proton exchange with the mineral surfaces causing enantiomerization. Construction of sorption isotherms revealed that the racemate and enantiomers have different isotherm shapes, possibly indicating different sorption mechanisms. Sorption was fit to the Freundlich model, with n_{2hr} being statistically different for the racemate and *S*-(-)-malathion and *R*-(*+*)- and *S*-(-)-malathion with bentonite, but not for calcite and montmorillonite. $K_{F,2hr}$ values were not significantly different for the racemate and enantiomers for bentonite, calcite, or montmorillonite, suggesting that the minerals had the same sorption capacity for the racemate or enantiomers.

Racemic metalaxyl and metalaxyl-M (consisting of 97% *R*-(-)-metalaxyl and 3% *S*-(*+*)-metalaxyl) were found to sorb to bentonite and montmorillonite, but sorption to calcite and kaolinite was not apparent. Sorption was fit to the Freundlich model with $n > 1$ and K_F indicating that bentonite had a higher sorption capacity than montmorillonite. Sorption of *R*-metalaxyl from metalaxyl-M was greater than racemic metalaxyl for both sorbents, but *S*-(*+*)-metalaxyl sorption was the greatest overall after consideration of the small amount of *S*-(*+*)-metalaxyl present in metalaxyl-M. Chiral analysis indicated that sorption of racemic metalaxyl was nonenantioselective with racemic EFs. However, sorption of metalaxyl-M resulted in an increase of EF with bentonite and montmorillonite indicating the possibility of enantioselective sorption. Solution EFs also increased for calcite, and at one concentration level EF decreased for kaolinite, suggesting that enantiomerization is occurring for metalaxyl-M when in contact with these sorbents, since sorption on calcite and kaolinite was not observed.

Although enantiomerization of chiral pesticides has been observed during degradation and in aprotic solvents, this research presents the first evidence of enantiomerization during sorption for two current-use chiral pesticides. The rapid enantiomerization of malathion and the enantiomerization and possible enantioselective sorption of metalaxyl to mineral surfaces questions the efficacy of using a chiral switch for these two pesticides. This research substantiates the need for enantiomer specific sorption analysis for chiral pesticides to understand their fate and effects in the environment.

DEDICATION

This dissertation is dedicated to my parents, Manuel and Gayle. Thank you for your unconditional love and giving me the courage to pursue my dreams.

ACKNOWLEDGMENTS

I would like to acknowledge my advisory committee for their insight and knowledge during my tenure as a doctoral student. Thank you to Dr. Wayne Garrison for support and helpful insight during research. To my advisor, Dr. Cindy Lee, I am grateful for her understanding, patience, and flexibility during my years as a graduate student. For statistical analysis, I am indebted to Dr. Julia Sharp.

I am grateful to Chiral Technologies for the donation of the chiral stationary phase. I would also like to acknowledge Nutra Manufacturing for laboratory use and financial support. Balancing my work and school lives have been a challenge and I owe thanks to my superiors and colleagues at Nutra Manufacturing. Specific thanks to Russ Reeves and Dr. Wendell Haymon for patience and flexibility. Also sincere thanks to Mindie Thompson for her constant support, encouragement, and laughter every day.

To my former research colleagues, Tess Brothersen and John Sivey, I owe sincere thanks for their support, encouragement, understanding, and long-distance friendship through the years. To Alicia Mathers I am indebted for friendship, encouragement, and understanding the demands of school and work simultaneously.

To my family, I am forever grateful for love, support, and encouragement. I would not be who I am today without their constant presence in my life. To my brother Brad, I am thankful for his unconditional love, support, and knowing that I am his favorite sister.

TABLE OF CONTENTS

Page

TITLE PAGE i

ABSTRACT ii

DEDICATION v

ACKNOWLEDGMENTS vi

LIST OF TABLES ix

LIST OF FIGURES x

CHAPTER

1. INTRODUCTION 1

Literature Review 3
Research Objectives 39
References 42

2. SORPTION OF RACEMIC MALATHION TO MINERALS 56

Abstract 56
Introduction 57
Materials and Methods 61
Results and Discussion 66
Conclusion 81
References 84

3. SORPTION AND ENANTIOMERIZATION OF MALATHION ENANTIOMERS TO MINERALS 88

Abstract 88
Introduction 89
Materials and Methods 92

Table of Contents (Continued)

Page

Results and Discussion 98
Conclusion 112
References 115

4. SORPTION OF RACEMIC METALAXYL AND METALAXYL-M TO MINERALS 119

Abstract 119
Introduction 120
Materials and Methods 123
Results and Discussion 129
Conclusion 142
References 144

5. CONCLUSIONS AND RECOMMENDATIONS 149

Conclusions 149
Recommendations for Future Work 152

APPENDICES

A. Supplementary Material for Chapter 2 156
B. Supplementary Material for Chapter 3 169
C. Supplementary Material for Chapter 4 182

LIST OF TABLES

Table		Page
1.1	Enantioselective sorption of amino acids on environmental surfaces	9
2.1	*Rac*-malathion Freundlich isotherm parameters	75
2.2	Bentonite Freundlich isotherm parameters of *rac*-malathion with sorbent weight	78
2.3	*Rac*-malathion Freundlich isotherm parameters comparison	79
3.1	Malathion Freundlich isotherm parameters	112
4.1	Metalaxyl Freundlich isotherm parameters	137
B.1	Statistical analysis of malathion Freundlich isotherm parameters	182
C.1	Sorption of *rac*-metalaxyl to 50 mg bentonite	183
C.2	Statistical analysis of metalaxyl Freundlich isotherm parameters	193

LIST OF FIGURES

Figure Page

2.1 *Rac*-malathion loss in water after two hours. Error bars represent standard deviations of triplicates. A "*" indicates that the difference is statistically significant ($p < 0.05$) 67

2.2 First-order degradation of *rac*-malathion in water. Samples 1, 2, and 3 represent individual test tubes. The linear regression was performed on all data points. 69

2.3 *Rac*-malathion change in enantiomeric fractions after incubation in water. Error bars represent standard deviations of triplicates. The change of EF was not significant from $t = 0$ to $t = 13$ d ($p = 0.38$) 70

2.4 *Rac*-malathion sorption isotherms. Dotted lines demonstrate isotherm shapes and do not represent model fits. Error bars represent standard deviations of triplicates. Sorption to kaolinite was negligible 73

2.5 *Rac*-malathion Freundlich sorption isotherms. Error bars represent standard deviations of triplicates. C_s = *rac*-malathion sorbed (mg/kg) concentration; C_w = *rac*-malathion aqueous concentration (mg/L) after two hours equilibration 75

2.6 Enantiomeric fractions of *rac*-malathion sorption. Error bars represent standard deviations of triplicates. 83

3.1 Change in enantiomeric fractions for malathion after incubation in water. Error bars represent standard deviations of triplicates 100

3.2 Degradation of malathion in water. Error bars represent standard deviations of triplicates 102

List of Figures (Continued)

Figure Page

3.3 Enantiomerization of **A**) *R*-(*+*)-malathion and **B**) *S*-(-)-malathion after contact with bentonite for two hours. Error bars represent standard deviations of triplicates 105

3.4 Enantiomerization of **A**) *R*-(*+*)-malathion and **B**) *S*-(-)-malathion after contact with calcite for two hours. Error bars represent standard deviations of triplicates 106

3.5 Enantiomerization of **A**) *R*-(*+*)-malathion and **B**) *S*-(-)-malathion after contact with bentonite for two hours. Error bars represent standard deviations of triplicates 107

3.6 Hypothetical enantiomerization mechanism of malathion 109

4.1 Enantiomeric fraction change of *rac*-metalaxyl and metalaxyl-M in water. Error bars represent standard deviations of triplicates. A "*" indicates that the difference is statistically significant ($p < 0.05$) from $t = 0$ d 131

4.2 Bentonite sorption isotherms of **A**) *rac*-metalaxyl and *R*-(-)-metalaxyl and **B**) *S*-(*+*)-metalaxyl. Dotted lines represent isotherm shapes and do not represent model fits. Error bars represent standard deviations of triplicates 133

4.3 Montmorillonite sorption isotherms of **A**) *rac*-metalaxyl and *R*-(-)-metalaxyl and **B**) *S*-(*+*)-metalaxyl. Dotted lines represent isotherm shapes and do not represent model fits. Error bars represent standard deviations of triplicates 134

4.4 Increases in enantiomeric fractions for metalaxyl-M after sorption. Error bars represent standard deviations of triplicates. A "*" indicates that the difference is statistically significant ($p < 0.05$) from metalaxyl-M standard prior to addition to the sorbent 139

4.5 Hypothetical enantiomerization mechanism of metalaxyl 142

List of Figures (Continued)

Figure Page

A.1 Concentration of *rac*-malathion in the aqueous phase versus time after contact with 50 mg of bentonite 157

A.2 Achiral HPLC chromatogram of malathion 157

A.3 Chiral HPLC chromatogram of *rac*-malathion. *R*-(*+*)-malathion elutes first, followed by *S*-(-)-malathion 158

A.4 HPLC achiral chromatograms of *rac*-malathion standard **A**) before and **B**) after two hours incubation and **C**) potential breakdown products malaoxon, isomalathion, and malathion monocarboxylic acid. The presence of an unknown peak at 2.5min in B indicates the malathion is possibly breaking down in water, but is not identified as one of the degradation products in C 159

A.5 Potential breakdown products of malathion in water. Adapted from Wolfe et al. (1975) 160

A.6 Bentonite sorption isotherm of *rac*-malathion. Dotted line demonstrates isotherm shape and does not represent a model fit 161

A.7 Montmorillonite sorption isotherm of *rac*-malathion. Dotted line demonstrates isotherm shape and does not represent a model fit 162

A.8 Seashells sorption isotherm of *rac*-malathion. Dotted line demonstrates isotherm shape and does not represent a model fit 163

A.9 Diatomaceous earth sorption isotherm of *rac*-malathion. Dotted line demonstrates isotherm shape and does not represent a model fit 164

List of Figures (Continued)

Figure Page

A.10 Calcite sorption isotherm of *rac*-malathion. Dotted line demonstrates isotherm shape and does not represent a model fit 165

A.11 Kaolinite sorption isotherm of *rac*-malathion. Dotted line demonstrates isotherm shape and does not represent a model fit. Kaolinite sorption was negligible as observed by negative sorbed concentration values 166

A.12 Kaolinite Langmuir sorption isotherm of *rac*-malathion. C_s = *rac*-malathion sorbed concentration (mg/kg); C_w = *rac*-malathion aqueous concentration (mg/L) after two hours equilibration 167

A.13 Bentonite sorption isotherms of *rac*-malathion with modified sorbent weight. Dotted lines demonstrate isotherm shapes and do not represent model fits. Error bars represent standard deviations of triplicates. 168

A.14 Bentonite Freundlich sorption isotherms of *rac*-malathion with modified sorbent weight. C_s = *rac*-malathion sorbed concentration (mg/kg); C_w = *rac*-malathion aqueous concentration (mg/L) after two hours equilibration. Error bars represent standard deviation of triplicates 169

B.1 First order degradation of A) *R*-(*+*)-malathion and B) *S*-(-)-malathion in water. Samples 1, 2, and 3 represent individual test tubes. The linear regression was performed on all data points 170

B.2 Enantiomerization in water after two hours for **A**) *R*-(*+*)-malathion and **B**) *S*-(-)-malathion. The scales for both figures have been altered for data clarity. Error bars represent standard deviations of triplicates. A "*" indicates that the difference is significantly different ($p < 0.05$) 171

List of Figures (Continued)

Figure Page

B.3 Loss of **A**) *R*-(*+*)-malathion and **B**) *S*-(-)-malathion in water after two hours. Error bars represent standard deviations of triplicates. A "*" indicates the difference is statistically significant ($p < 0.05$)..... 172

B.4 HPLC achiral chromatograms of **A**) malathion and potential breakdown products malaoxon, isomalathion, and malathion monocarboxylic acid and **B**) *R*-(*+*)-malathion water control after two hours. The presence of an unknown peak at 2.5 min in **B** indicates the malathion is possibly breaking down in water, but is not identified as one of the degradation products in **A**..... 173

B.5 Enantiomerization of **A**) *R*-(*+*)-malathion and **B**) *S*-(-)-malathion after immediate contact with bentonite. Error bars represent standard deviations of triplicates..... 174

B.6 Enantiomerization of **A**) *R*-(*+*)-malathion and **B**) *S*-(-)-malathion after immediate contact with sorbent. Error bars represent standard deviations of triplicates..... 175

B.7 Bentonite sorption isotherms of *rac*-, *R*-(*+*)-, and *S*-(-)-malathion. Dotted lines represent isotherm shapes and do not represent model fits. Error bars represent standard deviations of triplicates..... 176

B.8 Calcite sorption isotherms of *rac*-, *R*-(*+*)-, and *S*-(-)-malathion. Dotted lines represent isotherm shapes and do not represent model fits. Error bars represent standard deviations of triplicates..... 177

B.9 Montmorillonite sorption isotherms of *rac*-, *R*-(*+*)-, and *S*-(-)-malathion. Dotted lines represent isotherm shapes and do not represent model fits. Error bars represent standard deviations of triplicates..... 178

List of Figures (Continued)

Figure Page

B.10 Bentonite Freundlich sorption isotherms of *rac*-, *R*-(*+*)-, and *S*-(-)-malathion. C_s = malathion sorbed concentration (mg/kg); C_w = malathion aqueous concentration (mg/L) after two hours equilibration 179

B.11 Calcite Freundlich sorption isotherms of *rac*-, *R*-(*+*)-, and *S*-(-)-malathion. C_s = malathion sorbed concentration (mg/kg); C_w = malathion aqueous concentration (mg/L) after two hours equilibration..... 180

B.12 Montmorillonite Freundlich sorption isotherms of *rac*-, *R*-(*+*)-, and *S*-(-)-malathion. C_s = malathion sorbed concentration (mg/kg); C_w = malathion aqueous concentration (mg/L) after two hours equilibration 181

C.1 Achiral HPLC chromatogram of metalaxyl 183

C.2 Chiral HPLC chromatogram of *rac*-metalaxyl..... 184

C.3 Chiral HPLC Chromatogram of metalaxyl-M..... 185

C.4 Stability of *rac*-metalaxyl and metalaxyl-M in water..... 186

C.5 *Rac*-metalaxyl sorption controls in water. Error bars represent standard deviations of triplicates. A "*" indicates that the difference is statistically significant ($p < 0.05$)..... 187

C.6 Metalaxyl-M sorption controls in water. Error bars represent standard deviations of triplicates 188

C.7 Enantiomeric fractions of *rac*-metalaxyl sorption controls in water. Error bars represent standard deviations of triplicates 189

C.8 Enantiomeric fraction of metalaxyl-M sorption controls in water. Error bars represent standard deviations of triplicates 190

List of Figures (Continued)

Figure Page

C.9 Bentonite Freundlich sorption isotherms of *rac*-, *R*-(-)-, and *S*-(+)-metalaxyl. C_s = metalaxyl sorbed concentration (mg/kg); C_w = metalaxyl aqueous concentration (mg/L) after 24 hours equilibration 191

C.10 Montmorillonite Freundlich sorption isotherms of *rac*-, *R*-(-)-, and *S*-(+)-metalaxyl. C_s = metalaxyl sorbed concentration (mg/kg); C_w = metalaxyl aqueous concentration (mg/L) after 24 hours equilibration 192

C.11 Enantiomeric fractions of *rac*-metalaxyl after sorption. Error bars represent standard deviations of triplicates 194

CHAPTER 1

INTRODUCTION

The current consensus in environmental science is that enantioselectivity only occurs via biological processes and abiotic processes such as sorption is nonenantioselective. Nonetheless, several environmental abiotic matrices are chiral such as quartz and kaolinite or can exhibit chirality such as calcite, montmorillonite, and organic matter. Interaction of a chiral molecule with a chiral surface could theoretically induce an enantiospecific process. Notably, enantiospecific analysis is achieved using the concept of chiral sorption by utilizing chiral stationary phases that interact differently with enantiomers and thereby separating enantiomers for chromatographic analysis. As seen with amino acid laboratory experiments and computational modeling, chiral sorption can occur. Disappearance of pesticide enantiomers in the environment is not always due to biotic processes because pesticides can significantly sorb to environmental matrices. Due to the small amount of research focused on enantiomer specific sorption, research is needed to understand what factors influence chiral sorption. To date, chiral sorption is predominately used as an investigative tool to understand homochirality and the origins of life. Accordingly, environmental science should investigate chiral sorption as a possible mechanism for the fate of organic contaminants. Without considering chiral sorption, a key environmental process could be overlooked.

In environmental science, sorption of pesticides from the aqueous phase to environmental matrices is instrumental in understanding environmental processes such as degradation, up-take by organisms, and transport. Yet we have much to learn about the mechanisms of sorption, especially for chiral pesticides in current use in the US. About 25% of pesticides used currently are chiral (Garrison, 2006). Sorption greatly alters the fate of pesticides in the environment in several ways. Reduction in bioavailability of pesticides affects uptake and biotransformation, which in turn could prevent microbial degradation. Oxidation, reduction, and hydrolysis reactions may vary for a sorbed pesticide versus one in aqueous solution due to different chemical properties such as pH and redox conditions for each phase. Furthermore, degradation, either biotic or abiotic, could reduce or increase toxicity by producing a product that is less toxic or more toxic than the original pesticide. Sorption also affects pesticide mobility; retardation of a pesticide can prevent further contamination of surface or ground water (Karickhoff, 1984; Schwarzenbach et al., 2003). Sorption of a pesticide to insoluble residues could result in complete removal of the pesticide by sedimentation or filtration (Bollag et al., 1992).

The extent of sorption of pesticides to environmental matrices is dependent on the properties of the sorbate and sorbent. Solubility, size, configuration, polarity, molecular structure, and chemical reactivity are a few properties of the sorbate and sorbent that affects sorption processes. Chirality does not affect many of the chemical and physical properties of pesticides, but the behavior of the enantiomers can be

affected when interacting with asymmetric entities such as enzymes. Soils and sediments include organic matter, which is considered the most important factor in the sorption of non-ionic pesticides. However, in the absence of organic matter, or in the presence of limited amounts of it, clay mineral content and composition may significantly influence sorption of non-ionic pesticides. Sorption of ionic pesticides is primarily controlled by pH. Sorption by organic matter and inorganic clay mineral surfaces is strongly influenced by the pH of the solution, the zero point of charge of the sorbent, and the pK_a of the sorbate. At typical environmental pH values, organic matter is negatively charged due to carboxylic acid and phenolic groups, which encourages increased sorption of cationic species. However, anionic species can also sorb to organic matter, albeit to a lower extent. Clay minerals are similar in that their surfaces are able to display both cationic and anionic sites at the same time, the distribution of which is also a factor of solution pH (Schwarzenbach et al., 2003).

Literature Review

The purpose of this critical literature review is to provide a look at current chiral use pesticides, (defined as currently registered with the US EPA) and their sorption characteristics as well as investigating the possibility of chiral sorption by reviewing enantioselective degradation studies. As will be indicated in this review, there are only a few reports investigating sorption of separate enantiomers to environmental matrices and there is an obvious lack of controls to determine the impact of sorption and

possibility of chiral sorption during enantioselective degradation research. The concept of chiral sorption will be introduced and pesticides will be discussed by chemical class: chloroacetanilides, conazoles, imidazolinones, organophosphates, phenoxy acids, pyrethroids, and miscellaneous pesticides including ethofumesate, fipronil, and metalaxyl.

Chiral sorption

Approximately 25% of all pesticides contain chiral active ingredients, which are nonsuperimposable mirror images known as enantiomers. Enantiomers have identical chemical and physical properties, except for the rotation of the plane of polarized light, but may have different biological and toxicological properties. Therefore, enantioselectivity, the preference of one enantiomer versus another, can occur in a biological environment. The role of biological processes is assumed because enantiomers react identically in symmetrical environments but can react differently in asymmetrical environments such in the presence of chiral molecules (e.g. enzymes in a biotic environment). Since companies market the majority of chiral pesticides as racemates, equal amounts of both enantiomers, there is a need for enantiospecific research as to the fate, transport, and toxicity of the individual enantiomers. The general assumption in the environmental scientific community is that abiotic processes, such as sorption, are nonenantiospecific; that is enantiomers will react identically or sorb identically to a given sorbent. Kohler et al. (1997) stated that changes in the distributions of enantiomers “unequivocally indicate” that enantioselective

biodegradation occurred because physicochemical processes cannot change the distribution. Muller and Kolher (2004) indicated that their findings were evidence of biodegradation because "sorption to organic matter was of minor importance and should be nonenantioselective." Wong (2006) agreed that physicochemical processes such as sorption are "generally identical" for enantiomers. Others suggest the probability of sorption being enantioselective, but indicate it is an unlikely possibility. Lewis et al. (1999) while acknowledging that Zipper et al. (1998) ruled out enantioselective sorption point out that organic matter has its "genesis with living matter" and "may retain chiral centers capable of selectively binding specific enantiomers." Hegeman and Laane (2002) stated that abiotic transformations could be enantioselective given the proper chiral catalyst but that no reports of such processes in the environment have been made. Both Lewis et al. (1999) and Hegeman and Laane (2002) reference Zipper et al. (1998) as proof that chiral sorption is likely not to occur. In fact, most enantioselective biodegradation studies do not even consider sorption, therefore chiral sorption as a possible pathway for the dissipation of chiral pesticides would not be considered. In most cases, there are no sterile controls to assess the impact of sorption, whether or not it is enantioselective, and sorption of chiral pesticides is only studied with racemates using achiral analysis. The possibility of enantioselective sorption should be investigated when researching enantioselectivity in toxicity and degradation.

Chiral sorption is a phenomenon that has not received due attention in examining the fate of organic contaminants in environmental matrices. For chiral sorption to occur, the sorbent must display chirality as well as the sorbate. There are naturally occurring chiral surfaces abundant in the environment. Quartz is considered the most abundant naturally occurring mineral and is a helical arrangement of SiO_4 tetrahedra, which displays left- and right-handed helix structures resulting in chiral surfaces (Horvath and Gellman, 2003). Calcite is an achiral mineral, but contains chiral crystal faces, such as the rhombohedral space group R3c (Hazen and Sholl, 2003). Kaolinite is considered chiral with asymmetric triclinic crystals; however, montmorillonite does not have chiral surfaces, but can exhibit chirality in the stacking of its structural units (Julg and Ozias, 1988; Siffert and Naidja, 1992). Similarly, metals have achiral bulk crystal structures but are able to expose chiral surfaces. For example, Cu(643) surfaces are nonsuperimposable and display a clockwise or counterclockwise orientation, which are designated as $Cu(643)^S$ or $Cu(643)^R$ (Horvath and Gellman, 2003). Alternatively, an achiral surface can be transformed into a chiral surface by either templating or imprinting. Templating is the adsorption of a chiral organic ligand onto an achiral surface, creating a chiral sorption site, whereas imprinting is sorption of molecules that cause restructuring of the achiral surface into a chiral surface (Horvath and Gellman, 2003).

Analyses of enantiomers of chiral compounds are achieved using the concepts of chiral sorption. Enantioselectivity is due to selective interactions on a chiral stationary

phase in chromatography techniques, thereby separating the enantiomers and allowing for enantiospecific quantitation. The theory behind chiral separation methods is the requirement of an asymmetrical environment. Symmetrical environments do not differentiate between enantiomers; chiral molecules react identically. However, asymmetrical environments can cause specific interactions with enantiomers. For enantiospecific interactions to occur, at least three interaction points between the enantiomer and the chiral surface must occur simultaneously and with three separate substituents attached to the chiral center (Berthod, 2006; Fornstedt et al., 1998; Hazen and Sholl, 2003). These three interaction points may be due to steric interactions, formation of hydrogen bonds, or strong polar interactions. When enantiospecific sorption to a chiral surface occurs, different sorption free energies are observed with enantiomer pairs. The energy difference observed with enantiospecific interactions is typically very small, approximately 1 kilojoule per mole, which requires a homogeneous chiral surface (Gellman, 2010). If a chiral surface exposes sorption sites with different enantioselectivities, it is possible that no enantioselectivity will be observed (Gellman, 2010). Therefore, specific analytical methods are required for chiral analysis. In HPLC and GC analyses, chiral stationary phases contain chiral selectors that provide the asymmetric or chiral environment required for enantioseparations. Cyclodextrins are commonly used as the chiral selectors; for example, β-cyclodextrin is commonly bound to the stationary phase in commercial GC columns. β-cyclodextrin is a cyclic, torus

shaped molecule that contains 35 stereogenic centers, providing a substantial chiral surface for enantioselectivity (Beesley and Scott, 1988).

Homochirality is a major source of contention for origin-of-life research. Homochirality is the exclusive presence of one enantiomer in living organisms, for example L-amino acids and D-sugars. The origin of homochirality is vigorously debated with two main theories: life on Earth originally existed with racemates and achiral molecules and as a result, homochirality arose during biological evolution; or conversely, homochirality existed before living organisms existed (Castro-Puyana et al., 2008). To investigate the question of homochirality, there have been several studies of the sorption of amino acid enantiomers to chiral surfaces. Chiral sorption has predominately remained a focus of homochirality investigations with little attention in the environmental fate of organic contaminants (Matallo et al., 1998, Oravec et al., 2010; Zipper et al., 1998).

Sorption of amino acids to mineral surfaces has been researched extensively, as indicated in Table 1.1. Researchers have studied the enantioselective sorption of amino acids by clay minerals such as bentonite and montmorillonite. Bondy and Harrington (1979) found that sorption of L-leucine, L-aspartic acid and D-glucose was significantly higher than their respective enantiomers on bentonite. The L- and D-forms of amino acids and sugars, respectively, are the most biologically common forms found in nature and selective sorption could explain the primary existence in these forms. However,

Table 1.1: Enantioselective sorption of amino acids on environmental surfaces

Sorbent	Amino Acid	Reference
Bentonite	Aspartic Acid	Bondy and Harrington, 1979
	Leucine	Bondy and Harrington, 1979
Calcite	Aspartic Acid	Hazen et al., 2001
Kaolinite	Arginine	Wedyan and Preston, 2005
	Aspartic Acid	Wedyan and Preston, 2005
	Glutamic Acid	Wedyan and Preston, 2005
	Isoleucine	Wedyan and Preston, 2005
	Leucine	Wedyan and Preston, 2005
	Methionine	Wedyan and Preston, 2005
	Phenylalanine	Wedyan and Preston, 2005
	Serine	Wedyan and Preston, 2005
	Valine	Wedyan and Preston, 2005
Montmorillonite	Alanine	Wedyan and Preston, 2005
	Arginine	Wedyan and Preston, 2005
	Aspartic Acid	Wedyan and Preston, 2005
	Glutamic Acid	Wedyan and Preston, 2005
	Isoleucine	Wedyan and Preston, 2005
	Leucine	Wedyan and Preston, 2005
	Methionine	Wedyan and Preston, 2005
	Phenylalanine	Wedyan and Preston, 2005
	Serine	Wedyan and Preston, 2005
	Valine	Wedyan and Preston, 2005
Quartz	Alanine	Bonner et al., 1974
		Wedyan and Preston, 2005
		Han and Sholl, 2009
		Han and Sholl, 2010
	Arginine	Wedyan and Preston, 2005
	Asparagine	Wedyan and Preston, 2005
	Aspartic Acid	Wedyan and Preston, 2005
		Han and Sholl, 2010
	Cysteine	Han and Sholl, 2010
	Glutamic Acid	Wedyan and Preston, 2005
	Isoleucine	Wedyan and Preston, 2005
	Leucine	Wedyan and Preston, 2005
	Methionine	Wedyan and Preston, 2005
	Phenylalanine	Wedyan and Preston, 2005

Table 1.1: Enantioselective sorption of amino acids on environmental surfaces, cont.

Sorbent	Amino Acid	Reference
Quartz	Serine	Wedyan and Preston, 2005
		Han and Sholl, 2009
		Han and Sholl, 2010
	Valine	Wedyan and Preston, 2005
Sediment	Alanine	Wedyan and Preston, 2005
	Arginine	Wedyan and Preston, 2005
	Aspartic Acid	Wedyan and Preston, 2005
	Glutamic Acid	Wedyan and Preston, 2005
	Isoleucine	Wedyan and Preston, 2005
	Leucine	Wedyan and Preston, 2005
	Methionine	Wedyan and Preston, 2005
	Phenylalanine	Wedyan and Preston, 2005
	Serine	Wedyan and Preston, 2005
	Valine	Wedyan and Preston, 2005

Siffert and Naida (1992) reported contrary evidence with D-aspartic acid and L-glutamic acid selectively sorbed to montmorillonite.

Enantioselective sorption of amino acids has also been studied using the crystal minerals quartz and calcite. Bonner et al. (1974) showed that L-alanine selectively sorbed to (-)-quartz while D-alanine selectively sorbed to (+)-quartz. However, they conducted these experiments in dimethylformamide. They found no significant sorption when water or alcohol was the solvent. They assumed that water was negatively affecting the sorption of alanine to quartz by competing for available sorption sites. Sorption of selected amino acids onto hydroxylated α-quartz was modeled using density functional theory (DFT) (Han and Sholl, 2009; 2010). Sorption of aspartic acid and asparagine on the hydroxylated α-quartz surface ($10\bar{1}0$) was found to be enantioselective (enantiomers not specified). Sorption of alanine on the (0001) and ($10\bar{1}0$) surfaces was found to be only weakly enantioselective. Cysteine enantioselectively sorbed to both surfaces, with enantioselectivity on the (0001) surface stronger than on the ($10\bar{1}0$) surface. However, serine significantly enantioselectively sorbed to both the (0001) and ($10\bar{1}0$) faces with opposite enantioselectivity per crystal face; the (0001) surface preferentially sorbed *S*-serine, while the ($10\bar{1}0$) surface preferentially sorbed *R*-serine. Hazen et al. (2001) investigated the sorption of aspartic acid to four different calcite crystals and found enantioselective sorption with two calcite crystals. They found the ($21\bar{3}1$) *R* type calcite face preferentially sorbed D-aspartic acid, while the mirror related ($31\bar{2}\bar{1}$) *L* type face preferentially sorbed L-aspartic

acid. However, when Asthagiri and Hazen (2007) used *ab initio* DFT calculations, they found negligible enantioselectivity for alanine on the chiral calcite ($21\bar{3}\bar{1}$) surface. They determined that the weak enantioselectivity was due to the inability of alanine to form three strong points of interaction, which as mentioned previously is a requirement for strong enantioselectivity.

Wedyan and Preston (2005) discovered significant enantioselectivity by an ashed sediment collected from an estuary. They found that aspartic acid, glutamic acid, serine, arginine, alanine, valine, methionine, phenylalanine, isoleucine and leucine exhibited significant enantioselectivity, with differences in preferred enantiomers. For example, the sediment preferentially adsorbed D-enantiomers of aspartic acid and leucine and L-enantiomers of glutamic acid and phenylalanine. Sorption of racemic amino acids to wet sediment and freeze dried sediment was inconclusive. For the ashed sediment, organic matter was removed by thermally treatment at 450˚C for 15 h, which as they point out likely altered the surfaces of the minerals. They also observed differences in enantioselectivity with size fraction and pH. Wedyan and Preston (2005) tested pure minerals (quartz, montmorillonite, and kaolinite) as well and found all to sorb a variety of amino acids enantioselectively. Their report indicates enantioselective sorption occurred for amino acids in natural sediments and mineral components, which suggests the possibility of enantioselective pesticide sorption to environmental surfaces.

Isomerization

Isomerization of chiral pesticides, which includes racemization, enantiomerization, and diastereomerization, is a concern that is commonly overlooked in environmental research. Definitions of isomerization terms are as follows: racemization is defined as an irreversible macroscopic process when an enantiopure compound is converted to the other enantiomer forming a racemic mixture; enantiomerization is the reversible microscopic process of enantiomer conversion, which can result in a racemic mixture, complete transformation from one enantiomer to the other, or partial enantiomerization resulting in different enantiomeric fractions; and diastereomerization is the interconversion of diastereoisomers (Reist et al., 1995; Wolf, 2008). Enantioselective toxicity research has shown that the toxicological activity of some pesticides resides primarily in one enantiomer and they could possibly be marketed as a "chiral switch", i.e. formulations containing only or mostly the active enantiomer. For example, the fungicidal activity of metalaxyl is entirely due to the *R*-enantiomer, therefore the use of metalaxyl-M (containing 97.5% *R*-metalaxyl and 3% of the inactive *S*-enantiomer) is used to reduce application rates of up to 50% in comparison to the racemate (Buser et al., 2002). If isomerization occurs with chiral pesticides applied as only one enantiomer, the effectiveness of the pesticide is reduced, indicating isomerization of chiral pesticides during sorption and degradation processes should be considered. Racemization and enantiomerization are commonly used as interchangeable terms, but in this dissertation, the term enantiomerization is used to

depict changes in enantiomeric composition, regardless of the formation of a racemate or other enantiomeric mixtures.

Imidazolinones

The imidazolinone herbicides are amphoteric, containing an imidazoline ring with an aromatic moiety containing a carboxylic acid functional group. All the imidazolinones are chiral, containing a chiral center on the imidazoline ring. Given their amphoteric nature and chirality, imidazolinones are candidates for enantioselective sorption given the research that shows such behavior by amino acids, many of which are amphoteric.

pH-dependent sorption of the imidazolinone herbicides to soils and soil components has been demonstrated, indicating soil pH as the most important factor in sorption processes due to the amphoteric nature of imidazolinones. As pH values overcome the point zero charge (PZC), soil components become negatively charged and repulsion occurs with the anionic form of the imidazolinones. However, when the pH falls below the PZC, the soil becomes positively charged and enhanced sorption occurs (e.g., Che et al., 1992; Loux et al., 1989; Pusino et al., 1997; Regitano et al., 1997; Rocha et al., 2002). Organic matter also plays an important role in the sorption of imidazolinones to soil; higher organic matter content leads to higher adsorption (e.g., Loux et al., 1989; Regitano et al., 1997; Regitano et al., 2000; Undabeytia et al., 2004).

Since organic matter could possibly contain several stereogenic centers, chiral sorption should not be ruled out as a possibility.

Imidazolinone sorption to kaolinite, hectorite, and illite has been found to be significant and dependent on pH (Che et al., 1992). Due to the chiral nature of clays, chiral sorption is possible, although Che et al. (1992) did not investigate enantioselectivity in their experiments. Cruz-Guzman et al. (2005) found imazethapyr sorption to bentonite negligible; however, substitution of the existing inorganic ions in clay minerals with organic cations resulted in an increase of imazethapyr sorption to bentonite. They used organic cations such as L-carnitine, L-cysteine ethyl ester and L-cystine dimethyl ester, which could possibly induce chiral sorption of imazethapyr to these organoclays. However, Cruz-Guzman et al. (2005) did not consider enantioselectivity in their study.

Investigations of the enantioselective degradation of imidazolinones found in the literature do not include soil analysis or any significant attempt to explain degradation behavior by ruling out sorption to the soils (Han et al., 2008; Jarman et al., 2005; Ramezani et al., 2010; Yi et al., 2007). Field studies of imazaquin and imazethapyr indicate enantioselective degradation occurred; however minimal soil analysis was performed since the field studies were method development applications for analysis of imidazolinones. Some researchers have suggested that abiotic degradation is slower than biodegradation, therefore dismissing sorption or other abiotic degradation as a possibility (Han et al., 2008; Yi et al., 2007). Ramezani et al. (2010) found that imazapyr,

imazethapyr, and imazaquin incubated in aerobic microcosms using six soils and the *R*-(*+*)-enantiomer of all three imidazolinones degraded faster than the *S*-(-)-enantiomers. They found the soil pH to be the main component affecting degradation rates. Soil pH ranged from 5.02 – 8.70, and as the pH increased, EFs for all three enantiomers increased from pH 5.02 – 7.60 and then decreased from 7.60 – 8.70. The authors discounted the other possible contributing factors, organic carbon and clay content, due to the similarity of the six soils, with the major variation being the soil pH. However, there is no conclusive evidence that the enantiomeric degradation observed is not due to chiral sorption because they did not include killed or autoclaved controls and other researchers (e.g., Ahmad and Rahman, 2009; Che et al., 1992) have demonstrated that sorption of these compounds are correlated to pH. Several soil components are chiral, or can exhibit chirality, such as quartz, calcite, goethite, kaolinite, and montmorillonite; as a result, chiral sorption is possible, as indicated by enantioselective sorption of amino acids to minerals and sediment (Wedyan and Preston, 2005).

Phenoxy acids

Mecoprop and dichlorprop are 2-phenoxy propionic acids that contain one asymmetric carbon, resulting in one enantiomer pair for each herbicide. As ionic pesticides, they share characteristics with amino acids, which have shown enantioselective sorption and enantiospecific sorption of mecoprop and dichlorprop has been reported in the literature. However, a frequently cited study found the sorption of

racemic mecoprop by aquifer material (sandstone and marl) and several minerals including amorphous SiO_2, $CaCO_3$, montmorillonite, γ-Al_2O_3, goethite, and MnO_2 to be nonenantioselective (Zipper et al., 1998). The only significant sorption of mecoprop occurred with SiO_2 and montmorillonite, which is attributable to their high specific surface areas. No enantiomeric ratios or fractions were provided, but the adsorption coefficients (K_d) confirmed the conclusion that enantioselective sorption was not responsible for changes in enantiomeric composition in groundwater samples collected from a landfill leachate plume. Importantly, this report has been used to prove the impossibility of chiral sorption in general (Hegeman and Laane, 2002; Lewis et al., 1999).

Matallo et al. (1998) reported that adsorption of racemic mecoprop and dichlorprop and the *R*-enantiomers on several calcareous soils was nonenantioselective. They used achiral liquid chromatography and assumed that the lack of differences in sorption for the racemic forms versus the *R*-enantiomers indicated no chiral sorption. The Freundlich distribution constant (K_F) for the *R*-enantiomers was essentially the same as the racemic forms. It is important to note that if the sorption of the racemates was primarily due to the *R*-enantiomers, sorption constants for both the racemates and the *R*-enantiomer would be the same. Notably, enantiomerization was not included as a possible hypothesis for the apparently identical sorption of the racemic phenoxy acids versus the *R*-enantiomers.

Oravec et al. (2010) investigated enantioselective sorption of mecoprop and its methyl ester to humic acid and ash extracted from a soil. Their results for mecoprop

showed slight enantioselectivity, which was dependent on the concentration of humic acid or ash. The EF was higher (EF = 0.52) at lower concentrations of humic acid but decreased to racemic (EF = 0.50) at higher concentrations. The opposite effect was observed with ash; the EF was lower (EF = 0.49) at lower concentrations and then increased to racemic (EF = 0.50) at higher concentrations. They reported that the methyl ester differences were significant but did not indicate what statistical tests were used.

Achiral mecoprop and dichlorprop sorption to environmental matrices that are potentially chiral has been reported frequently in the literature (Agertved et al., 1992; Clausen and Fabricius, 2001; Clausen et al., 2001; Felding, 1997; Ghoryashi and Bergstrom, 1991; Haberhauer et al., 2000; Madsen et al., 2000; Matallo et al, 1998; Riise and Salbu, 1992; Romero et al., 2001; Rugge et al., 1999; Thorstensen et al., 2001; Tuxen et al., 2000; Zipper et al., 1998). In these studies, the effects of pH, background electrolytes, and organic matter are the focus. Mecoprop did not sorb to quartz at pH 6.5, but when the pH was lowered to 2.4, significant sorption was observed (Clausen et al., 2001). In addition, Clausen et al. (2001) found significant sorption of mecoprop on calcite, with suspension pH below the PZC; however, no sorption occurred to kaolinite. They hypothesized that sorption of mecoprop occurred with electrostatic attraction of $SiOH_2^+$ in quartz, $Ca(OH_2)^+$ in calcite. In addition, they investigated the effect of the background electrolyte using calcium chloride in experiments with phenoxy acids sorption on iron oxides, quartz, calcite, kaolinite, and α-alumina (Clausen and Fabricius,

2001; Clausen et al., 2001). Clausen et al. (2001) observed increased sorption of mecoprop with increasing $CaCl_2$ for quartz (at pH 6.5) and kaolinite; however, they found the opposite effect for calcite with decreased sorption of mecoprop. They suggested that the competing effects of $CaCl_2$ increased competition for adsorption sites, increased aqueous complexation, and decreased activity of the charged ions. Clausen et al. (2001) suggested that increased sorption of mecoprop to quartz and kaolinite is attributed to Ca^{2+} sorbing onto silanol surface sites, therefore increasing the positively charged sites for the anionic mecoprop.

Organic matter also influences the sorption of mecoprop and dichlorprop to environmental surfaces; matrices with higher organic matter present in the soil result in higher sorption (Haberhauer et al., 2000; Riise and Salbu, 1992; Thorstensen et al., 2001). Mecoprop and dichlorprop have been shown to sorb to clay minerals and organic matter, which can exhibit chirality. Accordingly, enantioselective sorption should be considered when investigating the fate of the phenoxy acid herbicides.

Enantioselective degradation investigations of mecoprop and dichlorprop have resulted in significant enantioselectivity in field study and laboratory incubations (Garrison et al., 1996; Harrison et al., 2003; Jarman et al., 2005; Messina and Sinibaldi, 2007; Muller and Buser, 1997; Romero et al., 2001; Schneiderheinze et al., 1999; Williams et al., 2003; Zipper et al., 1999). In laboratory incubations, several reports indicate that the *S*-enantiomer is preferentially degraded; these incubations were predominately under aerobic conditions with soil (Harrison et al., 2003; Jarman et al.,

2005; Messina and Sinibaldi, 2007; Muller and Buser, 1997; Romero et al., 2001; Schneiderheinze et al., 1999; Williams et al., 2003; Zipper et al., 1999). Romero et al. (2001) observed differences in enantioselectivity when mecoprop and dichlorprop were incubated in a silt loam, sandy loam, and clay loam. The *S*-enantiomers of both pesticides were preferred for removal in the incubation in the clay loam, but the *R*-enantiomers were preferentially degraded in the silt loam and sandy loam (Romero et al., 2001). The *R*-enantiomers of dichlorprop were preferentially degraded in aerobic sediment incubations (Ludwig et al., 1992), in nitrate-reducing soil microcosms (Harrison et al., 2003; Williams et al., 2003), and in iron-reducing soil microcosms, but only after the addition of nitrate (Williams et al., 2003). In contrast, Harrison et al. (2003) found no enantioselectivity or degradation in incubations in sulfate reducing/methanogenic microcosms or sulfate reducing microcosms for mecoprop. In anaerobic microcosms incubated with racemic or separate enantiomers of mecoprop or dichlorprop, Zipper et al. (1999) observed no enantioselectivity or degradation. Notably, Ludwig et al. (1992) found no enantioselectivity or degradation of mecoprop in aerobic sediment microcosms, but significant enantioselectivity for dichlorprop occurred in the same microcosms.

In laboratory incubations with separate enantiomers, conflicting results are presented for enantioselectivity. In two cases, the *S*-enantiomers of mecoprop and dichlorprop were degraded faster independently of the initial form used for incubation: microcosms incubated with the *R*-enantiomers, enantiomerized to the *S*-enantiomer,

and then proceeded to degrade the *S*-enantiomers faster. However, the microcosms incubated with the *S*-enantiomers enantiomerized to the *R*-enantiomer, but continued to degrade the *S*-enantiomers faster (Messina and Sinibaldi, 2007; Muller and Buser, 1997). In contrast, Zipper et al. (1999) found that aerobic sewage sludge microcosms spiked with individual enantiomers of mecoprop and dichlorprop resulted in similar degradation rates for both enantiomers and no enantiomerization. In addition, sewage sludge that was pre-exposed to a specific enantiomer of mecoprop resulted in enantioselectivity of the same enantiomer. Sewage sludge pre-exposed with *R*-mecoprop selectively degraded *R*-mecoprop, but not *S*-mecoprop when incubated separately (Zipper et al., 1999).

Enantiomerization of phenoxy acids during incubation is important to consider when determining enantioselectivity, and in the literature, only a few authors investigated to determine its role in processing results (Harrison et al., 2003; Messina and Sinibaldi, 2007; Muller and Buser, 1997; Romero et al., 2001; Zipper et al., 1999). Several instances report enantiomerization of mecoprop and dichlorprop, with conversion of the *R*-enantiomers to the *S*-enantiomers and vice versa (Messina and Sinibaldi, 2007; Muller and Buser, 1997; Romero et al., 2001), but not in all cases (Harrison et al., 2003; Zipper et al., 1999). In the studies that did not investigate or consider enantiomerization, there may be significant bias in interpreting the enantioselectivity of phenoxy acids.

In reviewing the enantioselective degradation reports of mecoprop and dichlorprop, the authors either did not mention sorption or ruled it an abiotic process that does not affect enantioselectivity (Harrison et al., 2003; Garrison et al., 1996; Romero et al., 2001; Williams et al., 2003; Zipper et al., 1998). Curiously, not all of the reports of enantioselective degradation of phenoxy acids used controls to confirm this assumption (Jarman et al., 2005; Messina and Sinibaldi, 2007; Schneiderheinze et al., 1999; Wink and Luley, 1988). In most reports with sterile controls, no enantioselectivity or degradation was observed (Gu et al., 2008; Harrison et al., 2003; Ludwig et al., 2002; Williams et al., 2003; Zipper et al., 1999). In one case, enantioselectivity was not observed, but some degradation did occur for mecoprop and dichlorprop in the sterilized controls (Muller and Buser, 1997).

Pyrethroids

All pyrethroid pesticides are non-ionic and chiral with one to three asymmetric carbons, resulting in one to four pairs of optical isomers. Chirality in the pyrethroids can occur in the cyclopropyl ring (acid moiety), the α-cyano carbon (alcohol moiety), or both (Liu et al., 2008; Qin and Gan, 2007). Furthermore, each enantiomer pair has *cis* and *trans* diastereomers. In studying the environmental fate and processes of pyrethroids, there are two important caveats: enantiomerization and sorption to container walls during experimentation. Since all pyrethroids are chiral, it is critical to account for enantiomerization during chiral specific analyses due to possible bias in interpreting

results. Researchers report enantiomerization in several organic and aqueous/organic solvents. However, no pyrethroid enantiomerization has been shown in hexane, methylene chloride, and acetone; therefore, these solvents should be safe for use during chiral specific analyses (Li et al., 2010; Nillos et al., 2009; Qin and Gan, 2007). During experimentation, sorption of pyrethroids to container walls can be significant. For example, 25-65% of pyrethroids initially added to centrifuge tubes sorbed onto the glass walls (Oudou and Hansen 2002a, 2002b). As a result, correction for sorption to glassware must be accounted to reduce bias in interpreting data.

Non-ionic pyrethroids are highly hydrophobic with low water solubility and high octanol-water coefficients; therefore, sorption to soil and minerals is expected to be significant in reducing bioavailability and retarding degradation (Oudou and Hansen, 2002b). Studies show pyrethroids sorb to soils and sediments, with organic matter being the most influential factor (Delgado-Moreno et al., 2010; Ismail and Kalithasan, 2005; Kumari and Singh, 1993). Several studies show they sorb to quartz, corundum, kaolinite, montmorillonite, and goethite (Ali and Baugh, 2003; Ismail and Kalithasan, 2005; Kumari and Singh, 1993; Oudou and Hansen, 2002a; Oudou and Hansen, 2002b; Sharom and Solomon, 1981; Zhou et al., 1995). Given that minerals and organic matter can display chirality, and hydrophobic pyrethroids display significant sorption to these matrices, enantioselective sorption should be considered in the fate of these pesticides. Although enantioselective sorption of pyrethroids has not been reported, Oudou and Hansen (2002b) showed that quartz, corundum, goethite, and montmorillonite had

significantly higher sorption for one set of diastereomers of cypermethrin, while kaolinite had significantly higher sorption for another set of diastereomers. Selective diastereomer sorption is possible and is generally expected due to the difference in physical properties of diastereomers. However, enantioselective sorption should also be explored because the chiral nature of minerals could possibly induce chiral sorption.

Several studies exist of the enantioselective degradation of pyrethroids but few include sterilized controls or discussion of sorption as a potential removal mechanism. In one of the first investigations of enantioselective degradation of pyrethroids, Sakata et al. (1992) conducted aerobic microcosm incubations of permethrin, fenvalerate, cypermethrin, and deltamethrin to determine enantioselective degradation in soil. Although they did not calculate enantiomeric fractions or ratios, they observed obvious differences in degradation rates for each pyrethroid enantiomer. However, they did not use any sterile controls nor did they consider sorption to the soil or glassware. Liu et al. (2005) conducted field studies and laboratory incubations of *cis*-permethrin and *cis*-bifenthrin in sediments that indicated enantioselective degradation. Their field studies indicated preferential degradation of the (-) enantiomers, with ERs increasing from 1.0 at the surface to 1.32 and 1.54 at the subsurface for *cis*-bifenthrin and *cis*-permethrin, respectively. They postulate the differences in ERs at different depths are due to the more favorable conditions for microbial community to enantioselectively degrade the (-) enantiomers of *cis*-bifenthrin and *cis*-permethrin. Laboratory incubations indicated the same trend, with ERs decreasing with time of incubation. In a previous study, Liu et al.

(2004) reported laboratory incubations with cypermethrin indicated preferential degradation of the *trans* enantiomers and diastereomers. For both reports, there were no controls during the laboratory incubation experiments, and no discussion of abiotic processes, such as enantiomerization, sorption to glassware, or sorption to sediment being possible factors in interpreting the results.

Fenvalerate has two chiral centers, and therefore, has two pairs of enantiomers: α*R*,2*R* and α*S*,2*S*; α*R*,2*S* and α*S*,2*R*. Incubation of fenvalerate in a sandy clay loam resulted in changes to the enantiomer compositions after 45 days of incubation, with α*R*,2*R*-fenvalerate being the most persistent (Faraoni et al., 2004). Faraoni et al. (2004) proposed the changes were due either to enantiomerization or stereospecific hydrolysis of α*S*,2*R* and α*R*,2*S* to their free acid metabolites. They performed no mass balances to account for sorption to glassware, or to the soil. They also did not use killed controls or discuss microbial activity to account for the enantiomeric differences in fenvalerate.

Li et al. (2009) conducted incubation studies with fenpropathrin and fenvalerate in which they used sterile controls. They spiked alkaline soils separately with racemic fenpropathrin and racemic fenvalerate that resulted in significant degradation (90% and 75%, respectively) after 55 days, with slight enantioselectivity. Parallel incubation with soil sterilized by autoclaving resulted in no enantioselectivity and 10% disappearance of fenpropathrin and 8% of fenvalerate. Alkaline soils spiked with α*S*,2*S*-fenvalerate resulted in degradation and enantiomerization to the α*R*,2*S*-fenvalerate enantiomer. Li et al. (2009) also observed significant enantiomerization in the sterile controls as well as

18% loss of the α*R*,2*S*-fenvalerate initially added. Alkaline soil spiked with *S*-fenpropathrin and α*S*,2*R*-fenvalerate also resulted in significant enantiomerization in both the non-sterile and sterile soils. However, in acidic soils they found no evidence of enantiomerization of α*S*,2*S*-fenvalerate, *S*-fenpropathrin and α*S*,2*R*-fenvalerate and much slower degradation with very little degradation in the sterile soils. With further investigation, they found that the enantiomerization of *S*-fenpropathrin and α*S*,2*R*-fenvalerate was dependent on pH using sterilized methanol-buffer solutions. Enantiomerization of both was favored at alkaline pH. They observed interesting behavior for a product of the degradation of fenvalerate, fenvaleric acid, which is chiral. With the alkaline soil spiked with α*S*,2*S*-fenvalerate, *S*-fenvaleric acid formed, even though enantiomerization of α*S*,2*S*-fenvlerate to α*R*,2*S*-fenvalerate occurred. However, with alkaline soil spiked with racemic fenvalerate, both *S*- and *R*-fenvaleric acid formed. During the incubation periods, the ER of the produced fenvaleric acid was less than one and thereafter increased to greater than one, indicating reversed enantioselectivity, either due to enantioselective microbes or abiotic processes. However, there was no correction for sorption to glassware, and all activity in the sterile controls was assumed to be abiotic enantiomerization. They assumed that the observed enantiomerization of fenvalerate and fenpropathrin was chemically induced, but they did not investigate the role sorption may have played.

Aerobic and anaerobic incubation of bifenthrin, permethrin, cypermethrin, and cyfluthrin in soil and sediment was enantioselective (Qin et al., 2006). Enantioselective

degradation occurred for all pyrethroids in all live aerobic and anaerobic microcosms. Loss of pyrethroids occurred in all the sterile controls, but no enantioselectivity was observed, which is presumed by the authors to be a result of killing the microbial community. There was no accounting for sorption of pyrethroids to glassware, or mention of abiotic processes such as sorption being a factor in the disappearance of the pyrethroids during the incubation experiments. *Trans*-permethrin enantiomers degraded faster in aerobic soil microcosms and aerobic and anaerobic sediment microcosms (Qin and Gan, 2006). Permethrin degradation occurred faster in aerobic soils versus sediment and faster in aerobic sediment than in anaerobic sediment. Reversed enantioselectivity was found in the aerobic soil versus the anaerobic sediment microcosms. Again, there were no sterile controls, no accounting for sorption to glassware, and no mention of sorption as a possible mechanism for disappearance or change in enantiomeric composition. Hydrolysis of permethrin was put forth as a possible explanation for differences in enantiomer degradation rates; each enantiomer may undergo hydrolysis selectively at different rates during aerobic or anaerobic incubation. From the observance of degradation products, enantioselectivity of permethrin occurs not only in the parent enantiomers but also in formation of the immediate transformation products. Degradation of β-cypermethrin and β-cyfluthrin, which each has two enantiomer pairs, in aerobic soil microcosms resulted in faster degradation of the *trans*- enantiomers (Li et al., 2008). However, there were no sterile controls, no correction for sorption to glassware, and no mention of sorption.

Furthermore, enantiomerization was not indicated as a possible explanation of the enantioselectivity of β-cypermethrin and β-cyfluthrin in aerobic soil microcosms.

Organophosphorus pesticides

No reports of enantioselective sorption of organophosphorus pesticides have been published, although several reports discuss the importance of organic matter (Arienzo et al., 1993; Bondarenko and Gan, 2004; Ismail et al., 2002; Koleli et al., 2007; Kulluru et al., 2010; MacNamara and Toth, 1970; Pantelelis et al. 2006; Sujatha and Chacko, 1991; Yu and Zhou, 2005;) and clay minerals including kaolinite and montmorillonite (Gonzalez-Pradas et al., 1993; Iglesias-Jimenez et al., 1996; Mohan et al., 2001; Pal and Vanjara, 2001; Sanchez Camazano and Sanchez Martin, 1983). Currently, there is only one investigation of enantioselective degradation of a registered chiral organophosphorus pesticide, which is detailed in the malathion section below. Li et al. (2010) studied the enantiomerization of malathion and profenofos in several organic solvents and buffered aqueous solvents to determine chiral stability. Enantiomerization of profenofos did not occur in any solvent system. However, enantiomerization of malathion did occur in methanol, ethanol, and two aqueous buffers (pH 5.8 and 7.0), but not in hexane, isopropanol, acetone, and methylene chloride. Consequently, enantiomerization should be considered during enantiospecific analysis of malathion and perhaps other chiral organophosphorus pesticides.

Malathion

Malathion is an organophosphate chiral pesticide currently used in the United States as a broad spectrum insecticide. In 2000, approximately 11-13 million pounds and in 2006 approximately 15 million pounds of malathion were used annually (EPA, 2006). Malathion uses include agricultural, industrial, commercial, homeowner, and regional pest eradication programs and is also used for mosquito control due to its low mammalian toxicity and high toxicity to insects. Malathion is water soluble (aqueous solubility = 145 mg/L) and the phosphorothionate (P=S) moiety is easily oxidized to the oxon (P=O) resulting in malaoxon as the primary metabolite (Ballantyne, 2004). Malathion contains one chiral carbon, resulting in one enantiomer pair; however, malathion is marketed as a racemate. Toxicity research indicates that the *R*-(*+*)-enantiomer is more toxic than the respective *S*-(-)-enantiomer to rats, anthropods, and *Daphnia magna* (Polec et al., 1998; Zhang et al., 2011). Even though *R*-(*+*)-malathion appears to have more toxicity than the respective *S*-(-)-enantiomer, malathion has shown to racemize in protic solvents, as discussed previously, which would hinder the efficacy of applying enantiopure malathion in the environment (Li et al., 2010).

There are only a few reports of sorption of malathion to environmental matrices and no reports of enantioselective degradation (Bondarenko and Gan, 2004; Donia et al., 2011; Gonzalez-Pradas et al., 1993; Kulluru et al., 2010; MacNamara and Toth, 1970; Mohan et al, 2001; Pal and Vanjara, 2001; Sanchez-Camazano and Sanchez-Martin, 1983; Sujatha and Chacko, 1991; Uchimiya et al., 2012). Malathion is commonly

analyzed using spectrophotometric methods, which can be problematic due to matrix interferences from environmental samples. Sorption data produced from non-specific methods can result in biased data. Therefore, there is a need for malathion sorption research by a more specific method such as HPLC. Chiral analysis of malathion can be performed using HPLC coupled with a chiral column, and Ellington et al. (2001) successfully separated the enantiomers of malathion using a Chiralcel ®OJ column.

Sun et al. (2012) investigated the enantioselective degradation and chiral stability of malathion racemate and the individual enantiomers in soil and water samples. Degradation was found to follow first order kinetics, with *S*-(-)-malathion degrading more rapidly than *R*-(*+*)-malathion in all matrices. Interestingly, the degradation rates of the enantiomers were 1.5-2.5 times higher when applied individually compared to the racemic application. For soil, enantioselective dissipation was found to correlate with organic carbon content, indicating the higher the organic content the higher the increase in dissipation, but no correlation was found with the pH of the soils; however for water samples, alkaline pH was found to enhance enantioselective dissipation. Application of the individual enantiomers of malathion to soil and water resulted in enantiomerization to the opposite enantiomer, which confirms the enantiomerization seen in protic solvents by Li et al. (2010).

Chloroacetanilides

The chiral chloroacetanilides acetochlor and metolachlor have been found to sorb to soils, with organic matter the predominate factor determining sorption (e.g., Cristanto et al., 1995; Peter and Weber, 1985; Pusino et al., 1992; Wang et al., 1999). Acetochlor and metolachlor have also been found to sorb to montmorillonite, which can present chiral surfaces (Bosetto et al., 1994; Liu et al., 2002a; Liu et al., 2002b). However, there are only two reports of enantioselective degradation; but neither study adequately separated the enantiomers (Ma et al., 2006; Muller and Buser, 1995). Muller and Buser (1995) reported obvious enantioselectivity for metolachlor in both soil and sewage sludge, but their method did not sufficiently resolve the four enantiomers for quantitation. They also investigated the enantioselective degradation of acetochlor but the results were not reliable due to matrix interferences and incomplete resolution of the two enantiomers. They indicated evidence that the enantiomers of dimethanamid were not stable at the column temperatures used for their GC method. The provided chromatography of the chloroacetanilides in the incubation experiments was only appropriate for qualitative determination of enantioselectivity. Furthermore, they used no controls to rule out sorption, nor did they discuss sorption to the soil and sewage sludge as a possibility for the disappearance of the chloroacetanilides.

Ma et al. (2006) separately incubated racemic *rac*-metolachlor and *S*-metolachlor in live and sterilized soil. However, they were unable to resolve the enantiomers of metolachlor using a chiral column. They compared the degradation

between *rac*-metolachlor and *S*-metolachlor using an achiral column. In the live soil incubation, they found *S*-metolachlor degrades faster than *rac*-metolachlor. However, in the sterile soil, they observed decreases in the mass of each with a similar rate of loss for both *rac*- and *S*-metolachlor. The authors assumed that since the rate did not significantly differ in the sterile soil and the disappearance was much lower than in the live soil, that enantioselective degradation occurred in the live soil. This may be correct, but the lack of specific chiral analysis of the four metolachlor enantiomers and the disappearance of metolachlor in the sterile soil leads to the possibility that sorption may have occurred, and therefore, cannot be ruled out in this experiment. Li et al. (2010) did not observe enantiomerization of acetochlor in solvents such as hexane, methanol, ethanol, isopropanol, acetone, methylene chloride, and aqueous buffers. Acetochlor appears to be stable in solution, but enantiomerization of metolachlor in solution has not been studied. Acetochlor has one chiral carbon whereas metolachlor is axially chiral and has a chiral carbon. This difference in structure could affect enantiomerization in solution, which would affect interpretation of enantioselective biodegradation data.

Azoles

Several researchers have reported that azole pesticides sorb to soil and clay minerals (Andrades et al., 2004; Cadkova, et al. 2012; Marin-Benito et al., 2009; Riise et al., 2001; Singh, 2002; Thorstensen et al., 2001). Since soil components such as organic matter and clay minerals can display chirality, enantioselective sorption is possible.

There are several reports of enantioselective biodegradation of azoles in the literature. Microcosm incubation with racemic cyproconazole resulted in faster dissipation rates for diastereomers versus enantiomers, which Buerge et al. (2006) attributed to the differences in physical and chemical properties of diastereomers. Their enantioselectivity calculations also indicated enantioselective dissipation, with varied enantiomer preference. They indicated that while enantioselective sorption was possible, no reports with natural soils have appeared at the time of publication. They cited the experiments of Matallo et al. (1998) who reported that *rac*-mecoprop and *rac*-dichlorprop sorbed to the same extent as their *R*-enantiomers, but performed no chiral analysis. Buerge et al. (2006) determined diastereomerization did not occur through incubation of individual cyproconazole diastereomers; however, enantiomerization cannot be discounted since individual enantiomers were not incubated. The authors assumed that since the diastereomers did not convert, the enantiomers would not have converted. They used no controls during the laboratory incubation, which they justified by suggesting that keeping microcosms sterile for several months would be difficult, and that the dissipation of cyproconazole would be due to enantioselective biodegradation.

Propiconazole degradation in soil-water slurries was found to be nonenantioselective, but the diastereomers showed some slight selectivity (Garrison et al., 2011b). No loss of propiconazole was observed in sterile controls, so sorption was not a factor. Degradation of tebuconazole in soils was also found to be nonenantioselective, with only slight insignificant changes in EFs and no

enantioselectivity observed in sterile controls (Wang et al., 2012). Degradation of triadimefon in soils was found to be enantioselective; in Garrison et al. (2011a) the *S*(*+*)-enantiomer degraded faster, but in Li et al. (2011) the opposite effect was observed with the *R*(-)-enantiomer preferentially degraded. Sterile soils in the case of Garrison et al. (2011a) were nonenantioselective and a small amount of triadimenol, the microbial breakdown product of triadimefon, was observed. However, in Li et al. (2011), no triadimenol was formed but incubation of the individual triadimefon enantiomers resulted in enantiomerization, which was assumed to be solely abiotic. In fact, the racemization was much higher in the sterile controls versus the live samples. Li et al. (2012) also observed enantioselective degradation of fenbuconazole, with marked preference for the (-)-enantiomer, but no sterile controls were analyzed, so sorption could be a factor in enantioselectivity.

Miscellaneous pesticides

Ethofumesate

Several studies report that ethofumesate, an herbicide used to control certain grasses and broadleaf plants, sorbs to montmorillonite, humic acid, and soils (Azejjel et al., 2010; Chevillard et al., 2012; Igelsias-Jimenez et al., 1996; Sanchez-Martin et al., 1993; Santos-Buelga et al., 1992). However, only one study by Wang et al. (2005) report an investigation of enantioselective biodegradation of ethofumesate. In aerobic soil microcosms, (-)-ethofumesate degraded faster than (*+*)-ethofumesate. No controls

were used, nor was sorption mentioned as a possibility for the enantioselectivity of ethofumesate in the soils.

Fipronil

The phenylpyrazole insecticide fipronil is shown to sorb to soils predominately due to organic matter (Bobe et al., 1997; Doran et al., 2006; Lin et al., 2008; Lin et al., 2009; Masutti and Mermut, 2007; Mukherjee, 2006; Spomer and Kamble, 2010; Ying and Kookana, 2001). Enantioselectivity of fipronil enantiomers was varied for the matrices studied during enantioselective biodegradation experiments (Jones et al., 2007; Nillos et al., 2009; Tan et al., 2008).

The main factor influencing enantioselectivity was determined to be redox conditions. Jones et al. (2007) anaerobically incubated fipronil in two sediments with different redox conditions: sulfidogenic and methanogenic. Enantiomeric fractions indicated that the *S*-enantiomer degraded faster in the sulfidogenic sediment, with EF < 0.5. The EFs continued to decrease over time until the *R*-enantiomer began to be selectively dissipated, which caused the EF to slightly increase. However, EFs in the methanogenic sediment showed the opposite trend: the *R*-enantiomer was degraded faster, with EF > 0.5, and increased until the *S*-enantiomer began to dissipate, slightly decreasing the EF. Nillos et al. (2009) incubated fipronil in aerobic and anaerobic sediment microcosms and found that overall degradation was faster and enantioselectivity was more prevalent in the anaerobic sediments. In the aerobic

sediments, there was no consistent trend for enantioselectivity; one sediment selectively degraded the *R*-enantiomer faster, while the other two sediments selectively degraded the *S*-enantiomer faster. In anaerobic sediments, the *S*-enantiomer was degraded faster, and as with Jones et al. (2007), the EF decreased over time, until the *R*-enantiomer started to dissipate, thereby increasing the EF. The difference in the degradation and enantioselectivity in the anaerobic sediment was attributed to the redox potential, as with Jones et al. (2007). Tan et al. (2008) also incubated fipronil in aerobic and anaerobic conditions, but using soil and spiking separately with *rac*-, *R*-, and *S*-fipronil. Enantioselectivity was not found in the aerobic microcosms, but the concentration did decrease by 78%, indicating that both enantiomers disappeared at the same rate. However, microcosms incubated under flooded conditions displayed enantioselective degradation of the *S*-enantiomer. These microcosms were considered methanogenic, similar to Jones et al. (2007) but the enantioselectivity was the opposite. Microcosms spiked with the separate *R*- and *S*-enantiomers proved that enantiomerization did not occur, and therefore possible bias in interpreting the data from enantiomerization was ruled out. Controls were used in all experiments, and no enantioselectivity was observed, as predicted by the authors. Sorption was only mentioned by Tan et al. (2008) as a possibility as a reduction of overall fipronil concentration, but not for enantioselectivity during incubation.

Metalaxyl

The acetamide fungicide metalaxyl significantly sorbs to soils, montmorillonite, and kaolinite, with organic matter the predominate factor influencing sorption (e.g., Andrades et al., 2001; Andrades et al., 2004; Bermudez-Couso et al., 2011; Marin-Benito et al., 2012; Sharom and Edgington, 1982; Sukul and Spiteller, 2000). Significant enantioselective degradation of metalaxyl in field application and laboratory incubations has been reported, with varied enantioselectivity (Buerge et al., 2003; Buser et al., 2002; Chen and Liu, 2009; Jarman et al., 2005; Marucchini and Zadra, 2002; Monkiedje et al., 2003; Monkiedje et al., 2007; Muller and Buser, 1995).

The most significant correlation for enantioselectivity was pH. Buerge et al. (2003) used 20 soils to determine factors affecting enantioselectivity of metalaxyl during laboratory aerobic and anaerobic incubations. Significant correlation with soil pH was found for aerobic incubated soils: in soils with pH > 5, *R*-metalaxyl was degraded faster than *S*-metalaxyl, however at pH < 4; the enantioselectivity was reversed, with *S*-metalaxyl being degraded faster than *R*-metalaxyl. In soils at pH 4-5, no significant enantioselectivity was observed. In anaerobic soil incubations, no correlation with pH was found; of the six soils, four soils degraded *S*-metalaxyl, while one soil degraded *R*-metalaxyl and one showed no enantioselectivity. Jarman et al. (2005) found agreement with these pH trends; in soils with pH > 5 aerobically incubated with metalaxyl, significant enantioselective degradation for *R*-metalaxyl was found. In all of the reported enantioselective degradation studies of metalaxyl, none of the studies

mentioned sorption as a possibility and only a few studies used controls (Jarman et al., 2005; Marucchini and Zadra, 2002), while most did not (Buerge et al., 2003; Buser et al., 2002; Chen and Liu et al., 2001; Monkiedje et al., 2003; Muller and Buser, 1995). Jarman et al. (2005) used controls during incubation of metalaxyl and the controls were nonenantioselective; however, the total concentrations did decrease during incubation, which could be due to sorption and the sorption was nonenantioselective. Marucchini and Zadra (2002) only occasionally monitored the controls by achiral analysis and assumed that since the metalaxyl concentration did not decrease, the enantiomeric composition did not change; therefore no chiral analysis was performed on the controls. In comparison, significant dissipation was found for metalaxyl in other studies that used sterile controls, possibly indicating a role for sorption (Saha and Sukul, 1997; Sukul and Spiteller 2001).

Research Objectives

Enantioselectivity in environmental matrices is currently attributed solely to biological processes because of the theory that physical processes such as sorption are nonenantioselective. However, since environmental surfaces can display chirality, there is a need for investigation of enantioselective sorption to grasp the impact of chiral contaminants in the environment. Distinct mechanisms for the sorption or enantiomers versus the racemate are possible due to the presence of a chiral environment such as minerals. For chiral sorption to occur on a chiral surface, the three-point interaction model proposes that binding sites on the surface are selective to a specific enantiomer. Therefore, the chiral surface will selectively bind one enantiomer over another due to molecular interactions such as steric hindrance, hydrogen bonding, ionic and dipole interactions, and van der Waals interactions. These molecular interactions result in specific binding of an enantiomer to a chiral surface and no matter how the opposite enantiomer is rotated or positioned, three points of interactions are not achieved (Berthod, 2006). For example, chiral pesticides, such as mecoprop and dichlorprop, are known to undergo stereospecific interactions with enzymes, which are chiral and provide a surface for enantiospecific interactions to occur as reviewed by Muller and Kohler (2004). Since minerals can display chirality, enantioselectivity resulting in distinct sorption mechanisms for the racemate and enantiomers is possible.

The purpose of this research was to investigate enantioselective sorption of chiral current-use pesticides to minerals. Malathion was chosen for this research

because of the high volume of use in the United States, the lack of reliable sorption data due to the use of non-specific spectrophotometric methods, and the availability of a reliable chiral method for analyzing individual malathion enantiomers (Ellington et al., 2001). Metalaxyl was selected due to the availability of the racemate and metalaxyl-M, which consists primarily of the *R*-(-)-enantiomer and only a small amount of the *S*-(*+*)-enantiomer. Metalaxyl-M is used as a chiral switch and little research has been done in regards to its degradation and sorption mechanisms.

Individual enantiomer sorption of malathion and metalaxyl was investigated with mineral surfaces to determine if sorption was enantioselective. Sorption of individual enantiomers instead of only the racemate would eliminate competitive sorption between the two enantiomers and allow direct investigation of single enantiomer sorption. Using single enantiomers instead of only racemates would also elucidate the possibility of enantiomerization when the enantiomers are either in aqueous solution or in contact with the mineral surface, which would not be obvious in investigating sorption of only the racemic pesticide.

The following objectives were completed:

1. Investigated the sorption of racemic malathion to mineral surfaces to test the hypothesis that enantioselective sorption occurs.

2. Investigated the sorption of individual malathion enantiomers to mineral surfaces to test the hypothesis that the enantiomers show significant differences in sorption.

3. Investigated the sorption of racemic and individual enantiomers of metalaxyl to mineral surfaces to test the hypothesis that enantioselective sorption occurs and that there are significant differences in sorption for the racemate and enantiomers.

References

Agertved, J.; Rugge, K.; Barker, J. F. Transformation of the herbicides MCPP and atrazine under natural aquifer conditions. *Ground Water* **1992**, *30*, 500-506.

Ahmad, R.; Rahman, A. Sorption characteristics of atrazine and imazethapyr in soils of New Zealand: Importance of independently determined sorption data. *Journal of Agricultural and Food Chemistry* **2009**, *57*, 10866-10875.

Ali, M. A.; Baugh, P. J. Sorption—desorption studies of six pyrethroids and mirex on soils using GC/MS-NICI. *International Journal of Environmental Analytical Chemistry* **2003**, *83*, 923-933.

Andrades, M. S.; Sanchez-Martin, M. J.; Sanchez-Camazano, M. Significance of soil properties in the adsorption and mobility of the fungicide metalaxyl in vineyard soils. *Journal of Agricultural and Food Chemistry* **2001**, *49*, 2363-2369.

Andrades, M. S.; Rodriguez-Cruz, M. S.; Sanchez-Martin, M. J.; Sanchez-Camazano, M. Effect of the modification of natural clay minerals with hexadecylpyridinium cation on the adsorption—desorption of fungicides. *International Journal of Environmental Analytical Chemistry* **2004**, *84*, 133-141.

Arienzo, M.; Sanchez-Camazano, M.; Cristanto Herero, T.; Sanchez-Martin, M. J. Effect of organic cosolvents on adsorption of organophosphorus pesticides by soils. *Chemosphere* **1993**, *27*, 1409-1417.

Asthagiri, A.; Hazen, R. M. An ab inito study of adsorption of alanine on the chiral calcite surface. *Molecular Simulation* **2007**, *33*, 343-351.

Azejjel, H.; Ordax, J. M.; Draoui, K.; Rodriguez-Cruz, M. S.; Sanchez-Martin, M. J. Effects of cosolvents on the adsorption of ethofumesate by modified Moroccan bentonite and common clay. *Applied Clay Science* **2010**, *49*, 120-126.

Ballantyne, B. Toxicology of Fungicides. In *Pesticide Toxicology and International Regulation* **2004**, Marrs, T. C. and Ballantyne, B. Eds. John Wiley and Sons, Chichester, England.

Beesley, T. E.; Scott, R. P. *Chiral Chromatography* **1998**, John Wiley and Sons Ltd, Chichester, England.

Bermudez-Couso, A.; Fernandez-Calvino, D.; Pateiro-Moure, M.; Garrido-Rodriguez, B.; Novoa-Munoz, J. C.; Estevez, M. A. Adsorption and desorption behavior of metalaxyl in intensively cultivated acid soils. *Journal of Agricultural and Food Chemistry* **2011**, *59*, 7286-7293.

Berthod, A. Chiral recognition mechanisms. *Analytical Chemistry* **2006**, *78*, 2093-2099.

Bobe, A.; Coste, C. M.; Cooper, J. F. Factors influencing the adsorption of fipronil on soils. *Journal of Agricultural and Food Chemistry* **1997**, *45*, 4861-4865.

Bollag, J. M.; Meyers, C. J.; Minard, R. D. Biological and chemical interactions of pesticides with soil organic matter. *Science of the Total Environment* **1992**, *123/124*, 205-217.

Bondarenko, S.; Gan, J. Degradation and sorption of selected organophosphate and carbamate insecticides in urban stream sediments. *Environmental Toxicology and Chemistry* **2004**, *23*, 1809-1814.

Bondy, S. C.; Harrington, M. E. L-Amino acids and D-glucose bind stereoselectively to a colloidal clay. *Science* **1979**, *203*, 1243-1244.

Bonner, W. A.; Kavasmaneck, P. R.; Martin, F. S. Flores, J. T. Asymmetric adsorption of alanine by quartz. *Science* **1974**, *186*, 143-144.

Bosetto, M.; Arfaioli, P.; Fusi, P. Adsorption of metolachlor on homoionic montmorillonites. *Agrochimica* **1994**, *38*, 14-24.

Buerge, I. J.; Poiger, T.; Muller, M. D.; Buser, H. –R. Enantioselective degradation of metalaxyl in soils: Chiral preference changes with soil pH. *Environmental Science and Technology* **2003**, *37*, 2668-2674.

Buerge, I. J.; Poiger, T.; Muller, M. D.; Buser, H. –R. Influence of pH on the stereoselective degradation of the fungicides epoxiconazole and cyproconazole in soils. *Environmental Science and Technology* **2006**, *40*, 5443-5450.

Buser, H. –R.; Muller, M. D.; Poiger, T.; Balmer, M. E. Environmental behavior of the chiral acetamide pesticide metalaxyl: Enantioselective degradation and chiral stability in soil. *Environmental Science and Technology* **2002**, *36*, 221-226.

Cadkova, E., Komarek, M.; Kaliszova, R.; Koudelkova, V.; Dvorak, J.; Vanek, A. Sorption of tebuconazole onto selected soil minerals and humic acids. *Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes* **2012**, *47*, 336-342.

Castro-Puyana, M.; Salgado, A.; Hazen, R. M.; Crego, A. L. Alegre, M. L. M. The first contribution of capillary electrophoresis to the study of abiotic origins of homochirality: Investigation of the enantioselective adsorption of 3-carboxy adipic acid on minerals. *Electrophoresis* **2008**, *29*, 1548-1555.

Che, M.; Loux, M.M.; Traina, S.J.; Loga, T.J. Effect of pH on sorption and desorption of imazaquin and imazethapyr on clays and humic-acid. *Journal of Environmental Quality* **1992**, *21*, 698-703.

Chen, S.; Liu, W. Enantioselective degradation of metalaxyl in anaerobic activated sewage sludge. *Bulletin of Environmental Contamination and Toxicology* **2009**, *82*, 327-331.

Chevillard, A.; Angellier-Coussy, H.; Peyron, S.; Gontard, N.; Gastaldi, E. Investigating ethofumesate-clay interactions for pesticide controlled release. *Soil Science Society of America Journal* **2012**, *76*, 420-431.

Clausen, L.; Fabricius, I. Atrazine, isoproturon, mecoprop, 2,4-D, and bentazone, adsorption onto iron oxides. *Journal of Environmental Quality* **2001**, *30*, 858-869.

Clausen, L.; Fabricius, I.; Madsen, L. Adsorption of pesticides onto quartz, calcite, kaolinite, and α-alumina. *Journal of Environmental Quality* **2001**, *30*, 846-857.

Cristanto, T.; Sanchez-Camazano, M.; Arienzo, M.; Sanchez-Martin, M. J. Adsorption and mobility of metolachlor in surface horizons of soils with low organic matter content. *Science of the Total Environment* **1995**, *166*, 69-76.

Cruz-Guzman, M., Celis, R., Hermosin, M. C., Koskinen, W. C., and Cornejo, J. Adsorption of pesticides from water by functionalized organobentonites. *Journal of Agricultural and Food Chemistry* **2005**, *53,* 7502-7511.

Delgado-Moreno, L.; Wu, L.; Gan, J. Effect of dissolved organic carbon on sorption of pyrethroids to sediments. *Environmental Science and Technology* **2010**, *44*, 8473-8478.

Donia, A. M.; Atia, A. A.; Hussein, R. A.; Rashad, R. T. Efficient adsorption of malathion from different media using thermally treated kaolinite. *Desalination and Water Treatment* **2011**, *30*, 178-185.

Doran, G.; Eberbach, P.; Helliwell, S. The sorption and degradation of the rice pesticides fipronil and thiobencarb on two Australian rice soils. *Australian Journal of Soil Research* **2006**, *44*, 599-610.

Ellington, J. J.; Evans, J. J.; Prickett, K. B.; Champion Jr., W. L. High performance liquid chromatographic separation of enantiomers of organophosphorus pesticides on polysaccharide chiral stationary phases. *Journal of Chromatography A* **2001**, *928*, 145-154.

EPA. Reregistration eligibility decision for malathion **2006**, EPA 738-R-06-030.

Faraoni, M.; Messina, A.; Polcaro, C. M.; Aturki, Z.; Sinibaldi, M. Chiral separation of pesticides by coupled-column liquid chromatography. Application to the stereoselective degradation of fenvalerate in soil. *Journal of Liquid Chromatography and Related Technologies* **2004**, *27*, 995-1012.

Felding, G. Pesticide adsorption as a function of depth below surface. *Pesticide Science* **1997**, *50*, 64-66.

Fornstedt, T.; Sajonz, P.; Guiochon, G. A closer study of chiral retention mechanisms. *Chirality* **1998**, *10*, 375-381.

Garrison, A. W.; Schmitt, P.; Martens, D.; Kettrup, A. Enantiomeric selectivity in the environmental degradation of dichlorprop as determined by high-performance capillary electrophoresis. *Environmental Science and Technology* **1996**, *30*, 2449-2455.

Garrison, A. W.; Avants, J. K.; Jones, W. J. Microbial transformation of triadimefon to triadimenol in soils: Selective production rates of triadimenol stereoisomers affect exposure and risk. *Environmental Science and Technology* **2011a**, *45*, 2186-2193.

Garrison, A. W.; Avants, J. K.; Miller, R. D. Loss of propiconazole and its four stereoisomers from the water phase of two soil-water slurries as measured by capillary electrophoresis. *International Journal of Environmental Research and Public Health* **2011b**, *8*, 3453-3467.

Gellman, A. J. Chiral surfaces: Accomplishments and challenges. *ACS Nano* **2010**, *4*, 5-10.

Ghoryashi, M.; Bergstrom, L. Equilibrium studies of the adsorption of dichlorprop on three Swedish soil profiles. *Swedish Journal of Agricultural Research* **1991**, *21*, 157-163.

Gonzalez-Pradas, E.; Villafranca-Sanchez, M.; Fernandez Perez, M.; Socias Viciana, M. Adsorption of malathion from aqueous solution on homoionic bentonite samples. *Agrochimica* **1993**, *37*, 104-110.

Gu, X.; Wang, P.; Liu, D.; Lu, Y.; Zhou, Z. Stereoselective degradation of diclofop-methyl in soil and Chinese cabbage. *Pesticide Biochemistry and Physiology* **2008**, *92*, 1-7.

Haberhauer, G.; Pfeiffer, L.; Gerzabek, M. H. Influence of molecular structure on sorption of phenoxyalkanoic herbicides on soil and its particle size fractions. *Journal of Agricultural Food and Chemistry* **2000**, *48*, 3722-3727.

Han, L.; Guo, B.; Feng, J.; Lu, X.; Lin, J.M. Study on the enantioselective degradation of imazethapyr in soil by CE. *Chromatographia* **2008**, *68*, 1071-1073.

Han, J. W.; Sholl, D. S. Enantiospecific adsorption of amino acids on hydroxylated quartz (0001). *Langmuir* **2009**, *25*, 10737-10745.

Han, J.W.; Sholl, D. S. Enantiospecific adsorption of amino acids on hydroxylated quartz ($10\bar{1}0$). *Physical Chemistry Chemical Physics* **2010**, *12*, 8024-8032.

Harrison, I.; Williams, G. M.; Carlick, C. A. Enantioselective biodegradation of mecoprop in aerobic and anaerobic microcosms. *Chemosphere* **2003**, *53*, 539-549.

Hazen, R. M.; Sholl, D. S. Chiral selection on inorganic crystalline surfaces. *Nature Materials* **2003**, *2*, 367-374.

Hazen, R. M.; Filley, T. R.; Goodfriend, G. A. Selective adsorption of L- and D-amino acids on calcite: Implications for biochemical homochirality. *Proceedings of the National Academy of Sciences* **2001**, *98*, 5487-5490.

Hegeman, W. J. M. and Laane, R. W. P. M. Enantiomeric enrichment of chiral pesticides in the environment. *Reviews of Environmental Contamination and Toxicology* **2002**, *173*, 85-116.

Horvath, J. D.; Gellman, A. J. Naturally chiral surfaces. *Topics in Catalysis* **2003**, *25*, 9-15.

Iglesias-Jimenez, E.; Sanchez-Martin, M. J.; Sanchez-Camazano, M. Pesticide adsorption in a soil-water system in the presence of surfactants. *Chemosphere* **1996**, *32*, 1771-1782.

Ismail, B. S.; Kalithasan, K. Adsorption, desorption, and mobility of permethrin in Malaysian Soils. *Journal of Environmental Science and Health Part B: Pesticides Food Contaminants and Agricultural Wastes* **2005**, *39*, 419-429.

Ismail, B. S.; Enoma, A. O.; Cheah, U. B.; Lum, K. Y.; Malik, Z. Adsorption, desorption, and mobility of two insecticides in Malaysian agricultural soils. *Journal of Environmental Science and Health Part B: Pesticides Food Contaminants and Agricultural Wastes* **2002**, *37*, 355-364.

Jarman, J. L.; Jones, W. J.; Howell, L. A.; Garrison, A. W. Application of capillary electrophoresis to study the enantioselective transformation of five chiral pesticides in aerobic soil slurries. *Journal of Agricultural and Food Chemistry* **2005**, *53*, 6175-6182.

Jones, W. J.; Mazur, C. S.; Kenneke, J. F.; Garrison, A. W. Enantioselective microbial transformation of the phenylpyrazole insecticide fipronil in anoxic sediments. *Environmental Science and Technology* **2007**, *41*, 8301-8307.

Julg, A.; Ozias, Y. Asymmetric adsorption of ethyliminium cation on kaolinite and L-homochirality of amino acids in proteins. *Journal of Molecular Structure (Theochem)* **1988**, *179*, 17-25.

Karickhoff, S. W. Organic pollutant adsorption in aqueous systems. *Journal of Hydraulic Engineering* **1984**, *110*, 707-735.

Kohler, H. P., Angst, W.; Giger, W. Kanz, C.; Muller, S.; Suter, M. Environmental fate of chiral pollutants – the necessity of considering stereochemistry. *Chimia* **1997**, *51*, 947-951.

Koleli, N.; Demir, A.; Arslan, H.; Kantar, C. Sorption behavior of methamidophos in a heterogeneous alluvial soil profile. *Colloids and Surfaces A: Physiochemical Engineering Aspects* **2007**, *301*, 94-99.

Kulluru, P.P.; Das, B. S.; Panda, R. K. Evaluation of sorption and leaching potential of malathion and atrazine in agricultural soils of India. *International Journal of Environmental Research* **2010**, *4*, 75-90.

Kumari, K.; Singh, R. P. Sorption thermodynamics of cypermethrin at high concentrations on some Indian soils. *International Journal of Environmental Analytical Chemistry* **1993**, *53*, 115-124.

Lewis, D, L.; Garrison, A. W.; Wommack, K. E.; Whittemore, A.; Steudler, P.; Mellilo, J. Influence of environmental changes on degradation of chiral pollutants in soils. *Nature* **1999**, *401*, 898-901.

Li, Y.; Dong, F.; Liu, X.; Xu, J.; Li, J.; Kong, Z.; Chen, X.; Zheng, Y. Environmental behavior of the chiral triazole fungicide fenbuconazole and its chiral metabolites: Enantioselective transformation and degradation in soils. *Environmental Science and Technology* **2012**, *46*, 2675-2683.

Li, Z. Y.; Zhang, Z. C. ; Zhang, L.; Leng, L. Stereo and enantioselective degradation of β-cypermethrin and β-cyfluthrin in soil. *Bulletin of Environmental Contamination and Toxicology* **2008**, *80*, 335-339.

Li, Z.; Zhang, Z.; Zhang, L.; Leng, L. Isomer- and enantioselective degradation and chiral stability of fenpropathrin and fenvalerate in soils. *Chemosphere* **2009**, *76*, 509-516.

Li, Z.; Wu, T.; Li, Q.; Zhang, B.; Wang, W.; Li, J. Characterization of racemization of chiral pesticides in organic solvents and water. *Journal of Chromatography A* **2010**, *1217*, 5718-5723.

Li, Z.; Zhang, Y.; Li, Q.; Wang, W.; Li, J.; Enantioselective degradation, abiotic racemization, and chiral transformation of triadimefon in soils. *Environmental Science and Technology* **2011**, *45*, 2797-2803.

Lin, K.; Haver, D.; Oki, L.; Gan, J. Persistence and sorption of fipronil degradates in urban stream sediments. *Environmental Toxicology and Chemistry* **2009**, *28*, 1462-1468.

Liu, W.; Gan, J.; Yates, S. R. Influence of herbicide structure, clay acidity, and humic acid coating on acetanilide herbicide adsorption on homoionic clays. *Journal of Agricultural and Food Chemistry* **2002a**, *50*, 4003-4008.

Liu, W.; Fang, Z.; Liu, H.; Yang, W. Adsorption of chloroacetanilide herbicides on soil and its components. III. Influence of clay acidity, humic acid coating and herbicide structure on acetanilide herbicide adsorption on homoionic clays. *Journal of Environmental Sciences* **2002b**, *14*, 173-180.

Liu, W.; Gan, J. J.; Lee, S.; Werner, I. Isomer selectivity in aquatic toxicity and biodegradation of cypermethrin. *Journal of Agricultural and Food Chemistry* **2004**, *52*, 6233-6238.

Liu, W.; Gan, J.; Schlenk, D.; Jury, W. A. Enantioselectivity in environmental safety in current chiral insecticides. *Proceedings of the National Academy of Sciences* **2005**, *102*, 701-706.

Liu, H.; Zhao, M. Zhang, C. Ma, Y.; L, W. Enantioselective cytotoxicity of the insecticide bifenthrin on a human amnion epithelial (FL) cell line. *Toxicology* **2008**, *253*, 89-96.

Loux, M.M.; Liebl, R.A.; Slife, F.W. Adsorption of imazaquin and imazethapyr on soils, sediments, and selected adsorbents. *Weed Science* **1989**, *37*, 712-718.

Ludwig, P.; Gunkel, W.; Huhnerfuss, H. Chromatographic separation of the enantiomers of marine pollutants. Part 5: Enantioselective degradation of phenoxycarboxylic acid herbicides by marine microorganisms. *Chemosphere* **1992**, *24*, 1423-1429.

Ma, Y.; Liu, W. P.; Wen, Y. Z. Enantioselective degradation of *rac*-metolachlor and *S*-metolachlor in soil. *Pedosphere* **2006**, *16*, 489-494.

MacNamara, G.; Toth, S. J. Adsorption of linuron and malathion by soils and clay minerals. *Soil Science* **1970**, *109*, 234-239.

Madsen, L.; Lindhardt, B.; Rosenberg, P.; Clausen, L.; Fabricius, I. Pesticide sorption by low organic sediments: A screening for seven herbicides. *Journal of Environmental Quality* **2000**, *29*, 1488-1500.

Marucchini, C.; Zadra, C. Stereoselective degradation of metalaxyl and metalaxyl-m in soil and sunflower plants. *Chirality* **2002**, *14*, 32-38.

Marin-Benito, J. M.; Sanchez-Martin, M. J.; Andrades, M. S.; Perez-Clavijo, M.; Rodriguez-Cruz, M. S. Effect of spent mushroom substrate amendment of vineyard soils on the behavior of fungicides: 1. Adsorption—Desorption of penconazole and metalaxyl by soils and subsoils. *Journal of Agricultural and Food Chemistry* **2009**, *57*, 9634-9642.

Marin-Benito, J. M.; Andrades, M. S.; Rodriguez-Cruz, M. S.; Sanchez-Martin, M. J. Changes in the sorption-desorption of fungicides over time in an amended sandy clay loam soil under laboratory conditions. *Journal of Soils and Sediments* **2012**, *12*, 1111-1123.

Masutti, C. S. M.; Mermut, A. R. Sorption of fipronil and its sulfide derivative by soils and goethite. *Geoderma* **2007**, *140*, 1-7.

Matallo, M. Romero, E. Sanchez-Rasero, F. Adsorption of mecoprop and dichlorprop on calcerous and organic matter amended soils. *Journal of Environmental Science and Health, Part B: Pesticides Food Contaminants and Agricultural Wastes* **1998**, *33*, 51-66.

Messina, A.; Sinibaldi, M. CEC enantioseparations on chiral monolithic columns: A study of the stereoselective degradation of (*R*/*S*)-dichlorprop [2-(2,4-dichlorophenoxy) propionic acid] in soil. *Electrophoresis* **2007**, *28*, 2613-2618.

Mohan, K. R.; Ramesh, A.; Seshaiah, K. Studies on adsorption of selected organophosphorus pesticides by clays. *Indian Journal of Chemical Technology* **2001**, *8*, 278-280.

Monkiedje, A.; Spiteller, M.; Bester, K. Degradation of racemic enantiopure metalaxyl in tropical and temperate soils. *Environmental Science and Technology* **2003**, *37*, 707-712.

Monkiedje, A.; Zuehlke, S.; Maniepi, S. J. N.; Spiteller, M. Elimination of racemic and enantioenriched metalaxyl based fungicides under tropical conditions in the field. *Chemosphere* **2007**, *69*, 655-663.

Muller, M. D.; Buser, H. –R. Environmental behavior of acetamide pesticide stereoisomers. 2. Stereo- and enantioselective degradation in sewage sludge and soil. *Environmental Science and Technology* **1995**, *29*, 2031-2037.

Muller, M. D.; Buser, H. –R. Conversion reactions of various phenoxyalkanoic herbicides in soil. 1. Enantiomerization and enantioselective degradation of the chiral 2-phenoxypropionic acid herbicides. *Environmental Science and Technology* **1997**, *31*, 1953-1959.

Muller, T. A.; Kohler, H.-P.E. Chirality of pollutants—effects on metabolism and fate. *Applied Microbiology and Biotechnology* **2004**, *64*, 300-316.

Mukherjee, I. Sorption of fipronil in tropical soils. *Bulletin of Environmental Contamination and Toxicology* **2006**, *76*, 334-340.

Nillos, M. G.; Qin, S.; Larive, C. Schlenk, D.; Gan, J. Epimerization of cypermethrin stereoisomers in alcohols. *Journal of Agricultural and Food Chemistry* **2009**, *57*, 6938-6943.

Oravec, M.; Simek, Z.; Holoubek, I. The effect of humic acid and ash on enantiomeric fraction change of chiral pollutants. *Colloids and Surfaces A: Physiochemical Engineering Aspects* **2010**, *359*, 60-65.

Oudou, H. C.; Hansen, H. C. B. Sorption of lambda-cyhalothrin, cypermethrin, deltamethrin and fenvalerate to quartz, corundum, kaolinite and montmorillonite. *Chemosphere* **2002a**, *49*, 1285-1294.

Oudou, H. C.; Hansen, H. C. B. Sorption of cypermethrin diastereoisomers to quartz, corundum, goethite, kaolinite and montmorillonite. *International Journal of Environmental Analytical Chemistry* **2002b**, *82*, 529-544.

Pal, O. R.; Vanjara, A. K. Removal of malathion and butachlor from aqueous solution by clays and organoclays. *Separation and Purification Technology* **2001**, *24*, 167-172.

Pantelelis, I.; Karpouzas, D. G.; Menkissoglu-Spiroudi, U.; Tsiropoulos, N. Influence of soil properties on the degradation and adsorption of the nematicide fosthiazate. *Journal of Agricultural and Food Chemistry* **2006**, *54*, 6783-6789.

Peter, C. J.; Weber, J. B.; Adsorption, mobility, and efficacy of alachlor and metolachlor as influenced by soil properties. *Weed Science* **1985**, *33*, 874-881.

Polec, I.; Cieslak, L.; Sledzinski, B.; Ksycinska, H. Simple syntheses of malathion and malaoxon enantiomers, and isomalathion diastereoisomers: Toxicity-configuration relationship. *Pesticide Science* **1998**, *53*, 165-171.

Pusino, A.; Petretto, S.; Gessa, C. Adsorption and desorption of imazapyr by soil. *Journal of Agricultural and Food Chemistry* **1997**, *45*, 1012-1016.

Qin, S.; Gan, J. Enantiomeric differences in permethrin degradation pathways in soil and sediment. *Journal of Agricultural and Food Chemistry* **2006**, *54*, 9145-9151.

Qin, S.; Budd, R.; Bondarenko, S.; Liu, W.; Gan, J. Enantioselective degradation and chiral stability of pyrethroids in soil and sediment. *Journal of Agricultural and Food Chemistry* **2006**, *54*, 5040-5045.

Qin, S.; Gan, J. Abiotic enantiomerization of permethrin and cypermethrin: Effects of organic solvents. *Journal of Agricultural and Food Chemistry* **2007**, *55*, 5734-5739.

Ramezani, M. K.; Oliver, D. P.; Kookana, R. S.; Lao, W.; Gill, G.; Preston, C. Faster degradation of herbicidally-active enantiomer of imidazolinones in soils. *Chemosphere* **2010**, *79*, 1040-1045.

Regitano, J.B.; Alleoni, L.R.F.; Vidal-Torrado, R.; Casagrande, J.C.; Tornisielo, V.L. Imazaquin sorption in highly weathered tropical soils. *Journal of Environmental Quality* **2000**, *29*, 894-900.

Regitano, J.B.; Bischoff, M.; Lee, L.S.; Reichert, J.M.; Turco, R.F. Retention of imazaquin in soil. *Environmental Toxicology and Chemistry* **1997**, *16*, 397-404.

Reist, M.; Testa, B.; Carrupt, P. A.; Jung, M.; Schurig, V. Racemization, enantiomerization, diastereomerization, and epimerization: Their meaning and pharmacological significance. *Chirality* **1995**, *7*, 396-400.

Riise, G.; Salbu, B. Mobility of dichlorprop in the soil-water system as a function of different environmental factors. I. A batch experiment. *Science of the Total Environment* **1992**, *123/124*, 399-409.

Rocha, W.S.D.; Regitano, J.B.; Alleoni, L.R.F.; Tornisielo, V.L. Sorption of imazaquin in soils with positive balance of charges. *Chemosphere* **2002**, *49*, 263-270.

Romero, E.; Matallo, M. B.; Pena, A.; Sanchez-Rasero, F.; Schmitt-Kopplin, Ph.; Dios, G. Dissipation of racemic mecoprop and dichlorprop and their pure *R*-enantiomers in three calcareous soils with and without peat addition. *Environmental Pollution* **2001**, *111*, 209-215.

Rugge, K.; Bjerg, P. L.; Mosbaek, H.; Christensen, T. H. Fate of MCPP and atrazine in an anaerobic landfill leachate plume (Grindsted, Denmark). *Water Research* **1999**, *33*, 2455-2458.

Sakata, S.; Mikami, N.; Yamada, H. Degradation of pyrethroid optical isomers in soils. *Journal of Pesticide Science* **1992**, *17*, 169-180.

Sanchez Camazano, M.; Sanchez Martin, M. J. Factors influencing interactions of organophosphorus pesticides with montmorillonite. *Geoderma* **1983**, *29*, 107-118.

Sanchez-Martin, M. J.; Gonazlez-Pozuelo, J. M.; Sanchez-Camazano, M. Adsorption of ethofumesate by agricultural and natural soils. *Weed Research* **1993**, *33*, 479-486.

Santos-Buelga, M. D.; Sanchez-Martin, M. J.; Sanchez-Camazano, M. Effect of dissolved organic matter on the adsorption of ethofumesate by soils and their components. *Chemosphere* **1992**, *25*, 727-734.

Saha, T.; Sukul, P. Metalaxyl—its persistence and metabolism in soil. *Toxicological and Environmental Chemistry* **1997**, *58*, 251-258.

Schneiderheinze, J. M.; Armstrong, D. W.; Berthod, A. Plant and soil enantioselective biodegradation of racemic phenoxyalkanoic herbicides. *Chirality* **1999**, *11*, 330-337.

Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M. *Environmental Organic Chemistry*. **2003**, 2nd Edition. John Wiley and Sons, Inc.

Sharom, M. S.; Edgington, L. V. The adsorption, mobility, and persistence of metalaxyl in soil and aqueous systems. *Canadian Journal of Plant Pathology* **1982**, *4*, 334-340.

Sharom, M. S.; Solomon, K. R. Adsorption—desorption, degradation, and distribution of permethrin in aqueous systems. *Journal of Agricultural and Food Chemistry* **1981**, *29*, 1122-1125.

Siffert, B.; Naidja, A. Stereoselectivity of montmorillonite in the adsorption and deamination of some amino acids. *Clay Minerals* **1992**, *27*, 109-118.

Singh, N. Sorption behavior of triazole fungicides in Indian soils and its correlation with soil properties. *Journal of Agricultural and Food Chemistry* **2002**, *50*, 6434-6439.

Spomer, N. A.; Kamble, S. T. Sorption and desorption of fipronil in Midwestern soils. *Bulletin of Environmental Contaminant and Toxicology* **2010**, *84*, 264-268.

Sujatha, C. H.; Chacko, J. Malathion sorption by sediments from a tropical estuary. *Chemosphere* **1991**, *23*, 167-180.

Sukul, P.; Spiteller, M. Sorption study on metalaxyl in soils of different physiochemical properties. *Fresenius Environmental Bulletin* **2000**, *9*, 701-710.

Sukul, P.; Spiteller, M. Influence of biotic and abiotic factors on dissipating metalaxyl in soil. *Chemosphere* **2001**, *45*, 941-947.

Sun, M; Liu, D.; Zhou, G.; Li, J.; Qiu, X.; Zhou, Z.; Wang, P. Enantioselective degradation and chiral stability of malathion in environmental samples. *Journal of Agricultural and Food Chemistry* **2012**, *60*, 372-379.

Tan, H.; Cao, Y.; Tang, T.; Qian, K.; Chen, W. L.; Li, J. Biodegradation and chiral stability of fipronil in aerobic and flooded paddy soils. *Science of the Total Environment* **2008**, *407*, 428-437.

Thorstensen, C. W.; Lode, O.; Eklo, O. M.; Christiansen, A. Sorption of bentazone, dichlorprop, MCPA, and propiconazole in reference soils in Norway. *Journal of Environmental Quality* **2001**, *30*, 2046-2052.

Tuxen, N.; Tuchsen, P. L. Rugge, K.; Albrechtsen, H. –J.; Bjerg, P. L. Fate of seven pesticides in an aerobic qualifier studied in column experiments. *Chemosphere* **2000**, *41*, 1485-1494.

Uchimiya, M.; Wartelle, L. H.; Boddu, V. M. Sorption of triazine and organophosphorus pesticides on soil and biochar. *Journal of Agricultural and Food Chemistry* **2012**, *60*, 2989-2997.

Undabeytia, T.; Sanchez-Verdejo, T.; Morillo, E.; Maqueda, C. Effect of organic amendments on the retention and mobility of imazaquin in soils. *Journal of Agricultural and Food Chemistry* **2004**, *52*, 4493-4500.

Wang, P.; Jiang, S. R.; Qiu, J. Wang, Q. X.; Wang, P.; Zhou, Z. Q. Stereoselective degradation of ethofumesate in turfgrass and soil. *Pesticide Biochemistry and Physiology* **2005**, *82*, 197-204.

Wang, Q.Q.; Liu, W.P. Correlation of imazapyr and desorption with soil properties. *Soil Science* **1999**, *164*, 411-416.

Wang, X.; Wang, X.; Zhang, H.; Wu, C.; Wang, X.; Xu, H.; Wang, X.; Li, Z. Enantioselective degradation of tebuconazole in cabbage, cucumber, and soils. *Chirality* **2012**, *24*, 104-111.

Wedyan, M.; Preston, M. R. Isomer-selective adsorption of amino acids by components of natural sediments. *Environmental Science and Technology* **2005**, *39*, 2115-2119.

Williams, G. M.; Harrison, I.; Carlick, C. A.; Crowley, O. Changes in enantiomeric fraction as evidence of natural attenuation of mecoprop in a limestone aquifer. *Journal of Contaminant Hydrology* **2003**, *64*, 253-267.

Wink, O.; Luley, U. Enantioselective transformation of the herbicides diclofop-methyl and fenoxaprop-ethyl in soil. *Pesticide Science* **1998**, *22*, 31-40.

Wolf, C. *Dynamic Stereochemistry of Chiral Compounds-Principles and Applications* **2008**, The Royal Society of Chemistry, Cambridge, United Kingdom.

Wong, C. S. Environmental fate processes and biochemical transformations of chiral emerging organic pollutants. *Annuals of Bioanalytical Chemistry* **2006**, *386*, 544-558.

Yi, F.; Guo, B.; Peng, Z.; Li, H.; Marriott, P.; Lin, J.M. Study of the enantioseparation of imazaquin and enantioselective degradation in field soils by CZE. *Electrophoresis* **2007**, *28*, 2710-2716.

Ying, G. G.; Kookana, R. S. Sorption of fipronil and its metabolites on soils from South Australia. *Journal of Environmental Science and Health Part B: Pesticides, Food Contaminants, and Agricultural Wastes* **2001**, 36, 545-558.

Yu, Y.; Zhou, Q. Adsorption characteristics of pesticides methamidophos and glyphosate by two soils. *Chemosphere* **2005**, *58*, 811-816.

Zhang, A.; Xie, X.; Ye, J.; Lin, C.; Hu, X. Stereoselective toxicity of malathion and its metabolites, malaoxon and isomalathion. *Environmental Chemistry Letters* **2011**, *9*, 369-373.

Zhou, J. L.; Rowland, S.; Fauzi, R.; Mantoura, F. C. Partition of synthetic pyrethroid insecticides between dissolved and particulate phases. *Water Research* **1995**, *29*, 1023-1031.

Zipper, C.; Suter, M, J.; Haderlein, S. B.; Gruhl, M.; Kohler, H. P. Changes in the enantiomeric ratio of (*R*)- to (*S*)-mecoprop indicate in situ biodegradation of this chiral herbicide in a polluted aquifer. *Environmental Science and Technology* **1998**, *32*, 2070-2076.

Zipper, C.; Bolliger, C.; Fleischmann, T.; Suter, M. J. –F.; Angst, W.; Muller, M. D.; Kohler, H. –P. Fate of the herbicides mecoprop, dichlorprop, and 2,4-D in aerobic and anaerobic sewage sludge as determined by laboratory batch studies and enantiomer-specific analysis. *Biodegradation* **1999**, *10*, 271-278.

CHAPTER 2

SORPTION OF RACEMIC MALATHION TO MINERALS

Abstract

Sorption of racemic malathion to mineral surfaces with two hr batch experiments was conducted using achiral and chiral high performance liquid chromatography (HPLC) analysis to investigate the possibility of enantioselective sorption and accurately quantify sorption using specific HPLC analysis instead of nonspecific spectrophotometric analysis. Chiral HPLC analysis was used to determine the enantiomeric fractions (EFs) of malathion. Sorption of racemic malathion was nonenantioselective for all sorbents and the β-cyclodextrin control (EF = 0.5) even though the sorbents used were chiral or can display chirality. Achiral HPLC analysis indicated significant sorption of malathion to all sorbents, with the exception of kaolinite, and a fit to the Freundlich sorption model. Sorption capacity of malathion, as determined by Freundlich constant $K_{F,2hr}$, decreased in the order of bentonite, montmorillonite, calcite, seashells, and diatomaceous earth. Freundlich n_{2hr} values, which are a measure of sorbate concentration dependence on sorption, were less than one for bentonite, calcite, and montmorillonite, indicating increased malathion concentration resulted in decreased sorption free energies. The opposite effect was seen with the biotic solids diatomaceous earth and seashells with n_{2hr} being greater than one.

Introduction

Chiral pesticides contain one or more enantiomer pairs, which can behave differently in environmental systems. Although enantiomers have identical chemical and physical properties, except for the rotation of the plane of polarized light, they may have different biological and toxicological properties. Approximately 25% of all pesticides are chiral and since the majority of chiral pesticides are marketed as racemates, there is a need for enantiospecific research as to the fate, transport, and toxicity of the individual enantiomers (Garrison, 2006). For example, malathion is an organophosphate chiral pesticide currently used in the United States as a broad spectrum insecticide and is sold exclusively as a racemate. The general assumption is that abiotic processes, such as sorption, are nonenantiospecific; that is, enantiomers will react identically or sorb identically to a given sorbent (Hegeman and Laane, 2002; Kohler et al., 1997; Lewis et al., 1999; Muller and Buser, 1997; Wong, 2006; Zipper et al. 1998). In fact, most enantioselective biodegradation studies do not consider sorption; therefore, chiral sorption as a possible pathway for the fate of chiral pesticides is not considered. The possibility of enantioselective sorption should be investigated when researching enantioselectivity in toxicity and degradation because it can ultimately affect the interpretation of toxicity and degradation data. For example, if the *R*-enantiomer of a chiral pesticide is responsible for the toxic activity and is enantioselectively sorbed to environmental matrices, the effectiveness of the *R*-enantiomer on the target organisms is reduced due to the reduction in bioavailability.

In addition, if enantioselective degradation of an enantiomer is assumed without investigating the possibility of enantioselective sorption, incorrect conclusions can be drawn by attributing enantioselective degradation processes to the disappearance of the enantiomer when in fact the enantiomer may still be present but selectively sorbed to the matrix.

For chiral sorption to occur, the sorbent must display chirality as well as the sorbate. For enantiospecific interactions to occur, at least three interaction points between the enantiomer and the chiral surface must occur simultaneously and with three separate substituents attached to the chiral center (Berthod, 2006; Hazen and Sholl, 2003). These three interaction points may be due to steric interactions, formation of hydrogen bonds, or strong polar interactions. When enantiospecific sorption to a chiral surface occurs, different sorption free energies are observed for each enantiomer. The energy difference observed with enantiospecific interactions is typically very small, approximately 1 kilojoule per mole, which requires a homogeneous chiral surface (Gellman, 2010). If a chiral surface exposes sorption sites with different enantioselectivities, it is possible that no enantioselectivity will be observed (Gellman, 2010). In the environment, quartz, calcite, kaolinite, and montmorillonite are among the many naturally occurring chiral surfaces. Quartz is a helical arrangement of SiO_4 tetrahedra, which displays left- and right-handed helix structures resulting in chiral surfaces (Horvath and Gellman, 2003). Calcite is an achiral mineral, but contains chiral crystal faces, such as the rhombohedral space group R3c (Hazen and Sholl, 2003).

Kaolinite is considered chiral with asymmetric triclinic crystals; however, montmorillonite does not have chiral surfaces, but can exhibit chirality in the stacking of its structural units (Julg and Ozias, 1988; Siffert and Naidja, 1992).

Emerging research in the study of homochirality (the exclusive presence of one enantiomer in living organisms) has demonstrated the enantioselective sorption of amino acids to chiral surfaces by laboratory and modeling experiments (Asthagiri and Hazen, 2007; Bondy and Harrington, 1979; Bonner et al., 1974; Han and Sholl, 2009, 2010; Hazen et al., 2001; Siffert and Naida, 1992). Wedyan and Preston (2005) investigated the sorption of amino acids to sediment and found significant enantioselective sorption of aspartic acid, glutamic acid, serine, arginine, alanine, valine, methionine, phenylalanine, isoleucine, and leucine, with differences in preferred enantiomers. Similarly, sorption of amino acids to quartz, montmorillonite, and kaolinite was found to be enantioselective (Wedyan and Preston, 2005). However, chiral sorption has predominately remained a focus of homochirality investigations with little attention in the environmental fate of organic contaminants (Matallo et al., 1998, Oravec et al., 2010; Zipper et al., 1998). A frequently cited study found the sorption of racemic mecoprop by aquifer material (sandstone and marl) and several minerals including amorphous SiO_2, $CaCO_3$, montmorillonite, γ-Al_2O_3, goethite, and MnO_2 to be nonenantioselective (Zipper et al., 1998). Matallo et al. (1998) reported that adsorption of racemic mecoprop and dichlorprop and the *R*-enantiomers on several calcareous soils was nonenantioselective. Oravec et al. (2010) investigated enantioselective sorption of

mecoprop and its methyl ester to humic acid and ash extracted from a soil. Their results for mecoprop showed slight enantioselectivity, which was dependent on the concentration of humic acid or ash.

Malathion contains one chiral carbon, resulting in one enantiomer pair with the *R*-(*+*)-enantiomer more toxic than the respective *S*-(-)-enantiomer (Polec et al., 1998; Zhang et al., 2011). Malathion is commonly analyzed by spectrophotometric methods, which are nonspecific and can be biased due to matrix interferences from environmental surfaces (e.g., Gonzalez-Pradas et al., 1993; Mohan et al., 2001). To analyze malathion accurately, an achiral high performance liquid chromatography (HPLC) method should be utilized. Sorption of malathion to minerals, soils, and sediments has been reported (Bondarenko and Gan, 2004; Gonzalez-Pradas et al., 1993; MacNamara and Toth, 1970; Mohan et al., 2001; Pal and Vanjara, 2001; Kulluru et al., 2010; Sanchez Camazano and Sanchez Martin, 1983; Sujatha and Chacko, 1991) and with the exception of Kulluru et al. (2010) all have used nonspecific spectrophotometric analysis. Currently, there is only one report of enantioselective degradation of racemic malathion and the individual enantiomers (Sun et al., 2012). To date, there are no reports of chiral analysis of malathion to determine if enantioselective sorption is a possibility. My research aims to use chiral analysis to determine the sorption effects on the individual malathion enantiomers and to provide more information about the sorption of malathion to minerals using achiral HPLC analysis for more accurate results.

Materials and Methods

Chemicals and materials

Racemic malathion (> 97%), malathion monocarboxylic acid (technical grade), malaoxon (94.3%), and isomalathion (100 µg/mL in hexane) were obtained from Chem Service (West Chester, PA). Acetone (reagent grade), acetonitrile (UV grade), and hexane (HPLC grade) were obtained from Fisher Chemicals (Fair Lawn, NJ). Reagent alcohol, which consisted of approximately 90% ethanol denatured with approximately 5% methanol and 5% isopropanol was obtained from Richard-Allan Scientific (Kalamazoo, MI). Deionized water (> 18 MΩ·cm) was used for all experiments. The chiral stationary phase, cellulose tris-(4-methylbenzoate), was donated by Chiral Technologies (West Chester, PA), and β-cyclodextrin was obtained from Sigma-Aldrich (St. Louis, MO). Calcite crystals (0.2-0.5 cm), were used as received from Alfa Aesar (Ward Hill, MA), and bentonite and diatomaceous earth were used as received from Spectrum Chemicals (New Brunswick, NJ). Kaolinite (KGa-1b) and montmorillonite (SWy-2) were used as received from the Clay Minerals Society (West Lafayette, IN). Seashells were collected from various locations along the eastern coast of the US, and crushed to coarse particles before use.

Malathion degradation

To accurately analyze small changes in concentration or EFs, malathion aqueous solutions at 10 mg/L were prepared by adding a solution of malathion in acetone to 50

mL glass centrifuge tubes, evaporating to dryness with nitrogen and reconstituting in 40 mL of deionized water. The experiment was conducted in triplicate, and the centrifuge tubes were placed on a wrist action shaker protected from light. The centrifuge tubes were sampled by removing 2 mL of malathion solution and following the achiral and chiral extractions for analysis listed below.

Sorption experiments

Batch sorption experiments were conducted, using initial aqueous concentrations at 5, 10, 25, 50, and 75% of the aqueous solubility of malathion (145 mg/L). Malathion concentration ranges were determined from a comprehensive literature review indicating that in malathion sorption experiments the initial aqueous concentrations ranged from 4-750 mg/L, therefore 5-75% of aqueous solubility of malathion was selected to fall within this range (Donia et al., 2011; Gonzalez-Pradas, et al., 1993; MacNamara and Toth, 1970; Mohan et al., 2001). A 5 mL aliquot of each aqueous solution was added to a Pyrex test tube containing 50 mg of the sorbent. To determine the optimal sorbent solution: mass ratio, 5, 12.5, or 25 mg of bentonite were used. Control samples using deionized water, β-cyclodextrin, or chiral stationary phase cellulose tris-(4-methylbenzoate) were prepared. For β-cyclodextrin, the sorbent weight was 1 g in order to be above the water solubility of the sorbent. All experiments were prepared in triplicate. Sorption equilibration time was determined by conducting a time study using concentrations of malathion ranging between 7-35 mg/L; after 24 hours,

malathion was not detectable in the aqueous phase for all sorbents, except for kaolinite (see Figure A.1). Therefore, the equilibration time of two hours was chosen for all experiments. Samples and controls were vortexed briefly, mixed on a wrist-action shaker for two hours, and the phases separated by centrifuging at 1000 rpm for 15 min. The concentration of the pesticide in the aqueous phase was analyzed by achiral analysis for quantitation and chiral analysis for enantiomeric fraction determination.

Achiral HPLC analysis

Reverse phase HPLC analysis coupled with UV spectrophotometer detection is a practical method for analysis of water soluble pesticides with UV absorbent structural groups. The UV absorption maximum for malathion is at 210 nm, which required careful selection of the mobile phase solvent to avoid UV interference. To minimize solvent interferences, acetonitrile was used as the organic solvent in the mobile phase due to its low absorptivity (190 nm); the absorptivity of methanol (205 nm) is too close to the absorptivity of malathion for accurate quantitative analysis. Analysis was performed on a Waters Alliance 2690 Separation Module with a 2996 photodiode array detector equipped with Empower Pro chromatography software (Waters Corporation, Milford, MA). The column used was a Zorbax SB-C18 rapid resolution column, 3.5 µm pore size, 4.6 x 100 mm (Agilent Technologies, Wilmington, DE). The mobile phase consisted of 50:50 acetonitrile and deionized water with a flow rate of 1.5 mL/min, 50 µL injection volume, and ambient column temperature. Malathion absorbance was measured at

210 nm and the run time was 10 min, with malathion eluting at approximately 5.6 min (Figure A.2). Malathion concentrations were calculated using a linear regression plot through the origin of six concentration levels ranging from 0.24 – 160 mg/L of malathion (r^2 = 0.9995-0.9999) with a limit of detection (LOD) of 0.028 mg/L. An aliquot of the aqueous layer of the sorption samples and controls was diluted with acetonitrile for a 1:1 mixture of acetonitrile and deionized water, filtered with a 0.45 µm hydrophobic PFTE syringe filter into a HPLC autosampler vial, and analyzed. Recoveries of malathion in water were > 96%, verifying the complete extraction of malathion. Achiral analysis was used for quantitative analysis of sorption, considered as a loss of malathion from solution, with no consideration of chirality.

Chiral HPLC analysis

Chiral HPLC analysis was used for identification of enantiomers and was adapted from Ellington et al. (2001) using a normal phase HPLC method coupled with UV detection and a chiral column. Analysis was performed on a Perkin Elmer Series 200 HPLC with UV detection equipped with TotalChrom 6.3.2 chromatography software (Perkin Elmer, Waltham, MA). The chiral column was a 4.6 mm x 250 mm Chiralcel® OJ® packed with cellulose tris-(4-methylbenzoate) coated on a 10 µm silica gel substrate (Chiral Technologies, West Chester, PA). The mobile phase was 90:10 hexane: reagent alcohol, at 0.9 mL/min, with a column temperature of 20^{o}C and a Peltier autosampler temperature of 10^{o}C. Injection volume was 100 µL, and malathion enantiomers were

analyzed at 210 nm, for a run time of 25 min. Malathion enantiomers were separated with baseline resolution, and elution consisted of *R*-(*+*)-malathion at approximately 11.3 min followed by *S*-(-)-malathion at approximately 13.7 min (Figure A.3). LOD for both *R*-(*+*)- and *S*-(-)-malathion was 0.9 mg/L. For chiral analysis, an aliquot of the aqueous layer was extracted with 800 µL of hexane and transferred to a HPLC autosampler vial for analysis. To determine if sorption of the pesticide racemate was enantioselective, enantiomeric fractions (EFs) were calculated by using the peak areas for the *R*-(*+*) and *S*-(-) malathion enantiomers using the following equation:

$$\mathrm{EF} = \frac{[\text{R-(+)}]}{[\text{R-(+)}] + [\text{S-(-)}]} \qquad 2.1$$

A significant deviation from EF = 0.5 will indicate enantioselective sorption; EF < 0.5 indicates *R*-(*+*)-malathion concentration has decreased, and EF > 0.5 indicates *S*-(-)-malathion concentration has decreased. Recovery samples of racemic malathion indicated no deviation from racemic (EF = 0.5). Racemic malathion standard was analyzed throughout chiral analysis to verify the method was accurate by observation of consistently racemic EFs. Chiral analysis was used to calculate EFs to determine if enantiomerization or enantioselective sorption occurred for malathion.

Statistical analysis

Statistical analysis was performed using single factor ANOVA in Microsoft Excel (Microsoft Office 2010), using $\alpha = 0.05$. P values are reported throughout the results and discussion and $p < 0.05$ is determined to be statistically significant whereas $p > 0.05$ is not statistically significant. EF results are presented with standard deviations whereas the rate constant, half-life, and Freundlich isotherm parameters are presented with standard error for linear regression analysis.

Results and Discussion

Malathion degradation and enantiomerization in water

Controls with no sorbent indicated significant loss of malathion ($p < 0.05$) in water after two hours for four of the five concentration levels (Figure 2.1). In several of the achiral HPLC chromatograms, an extra peak was present at approximately 2.5 min, which could possibly be a breakdown product of malathion (Figure A.4). The peak also appeared in some of the sorption samples, at approximately the same amount. To identify the unknown peak, standard injections of malathion monocarboxylic acid, isomalathion, and malaoxon were analyzed. Comparison of retention times indicated the unknown peak was not one of these breakdown products, although there are other potential breakdown products of malathion degradation in water as presented in Figure A.5 (Wolfe et al., 1975). These other breakdown products of malathion were not analyzed since they are not available commercially. Degradation of malathion over 13

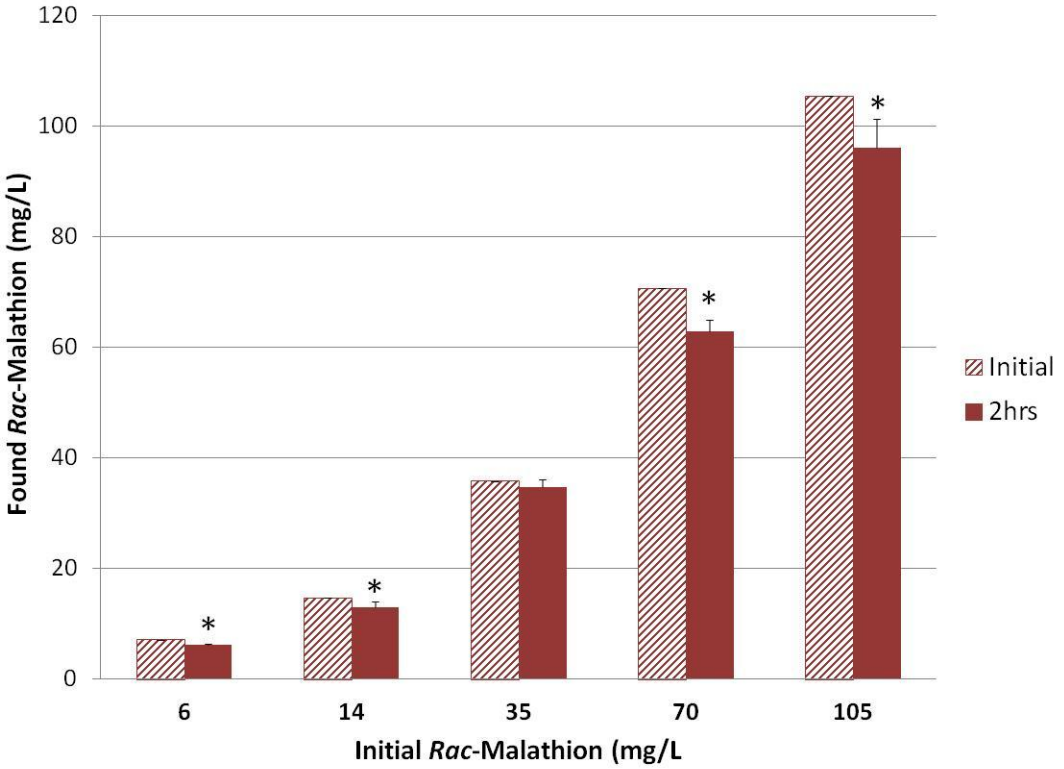


Figure 2.1: *Rac*-malathion loss in water after two hours. Error bars represent standard deviations of triplicates. A "*" indicates that the difference is statistically significant ($p < 0.05$).

days followed first-order kinetics, and the rate constant k and the half-life $t_{1/2}$ where determined by regression plot of ln (C_t) versus time (t) (Figure 2.2). using the following equations:

$$\ln (C_t) = -kt + \ln (C_o) \qquad 2.2$$

$$t_{1/2} = \ln (0.693/k) \qquad 2.3$$

where C_t is the concentration of malathion at time t (mg/L) and C_o is the initial concentration of malathion. The coefficient of determination was $r^2 = 0.6045$, which is low due to the study being conducted in triplicate (three separate test tubes containing malathion in water) which resulted in different kinetics for each test tube. The rate constant was $k = 0.107$ (± 0.016) d^{-1} and the $t_{1/2} = 6.48$ (± 0.97) d.

Sun et al. (2012) observed degradation of the racemate and individual enantiomers of malathion in aqueous environmental samples, however with higher rate constants ranging from 0.2114-0.8305 d^{-1} and shorter half-lives of $t_{1/2}$= 0.77-3.94 d. This difference in rates and half-lives could be accounted for because my research used deionized water, whereas Sun et al. (2012) used environmental water samples, which could attribute to faster degradation of malathion.

In addition, my study showed an insignificant ($p = 0.38$) decrease in EF, from EF = 0.50 (± 0.01) at t = 0 d to EF = 0.41 (± 0.11) at t = 13 d (see Figure 2.3). In the two hour

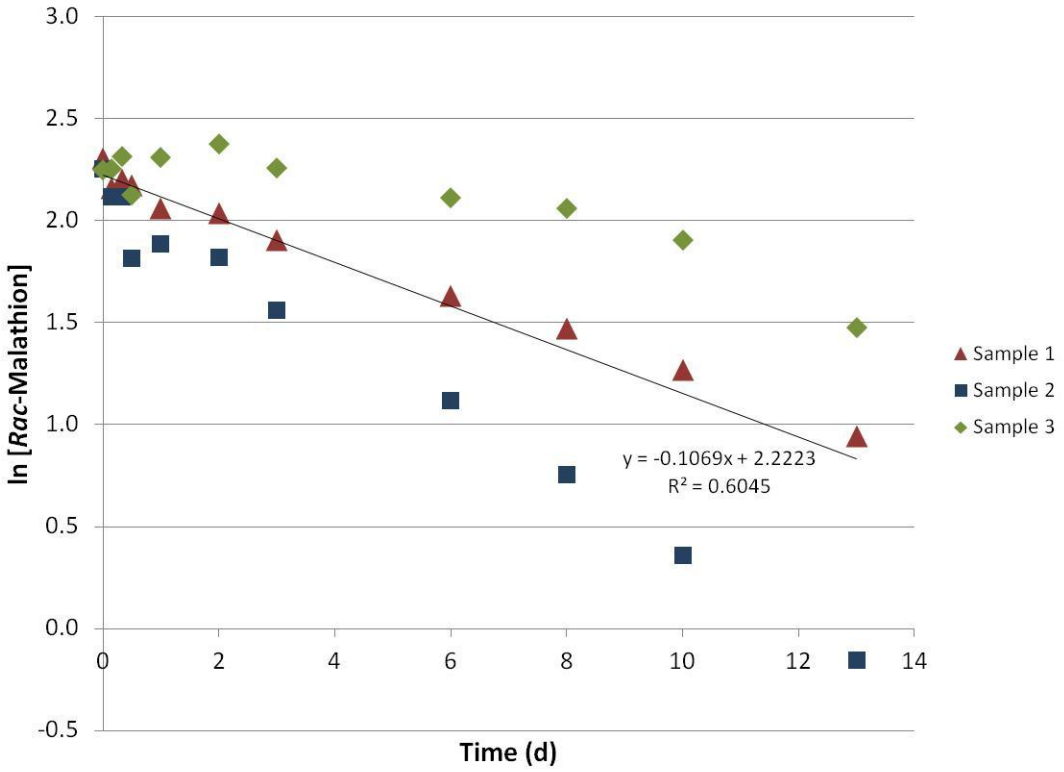


Figure 2.2: First-order degradation of *rac*-malathion in water. Samples 1, 2, and 3 represent individual test tubes. The linear regression was performed on all data points.

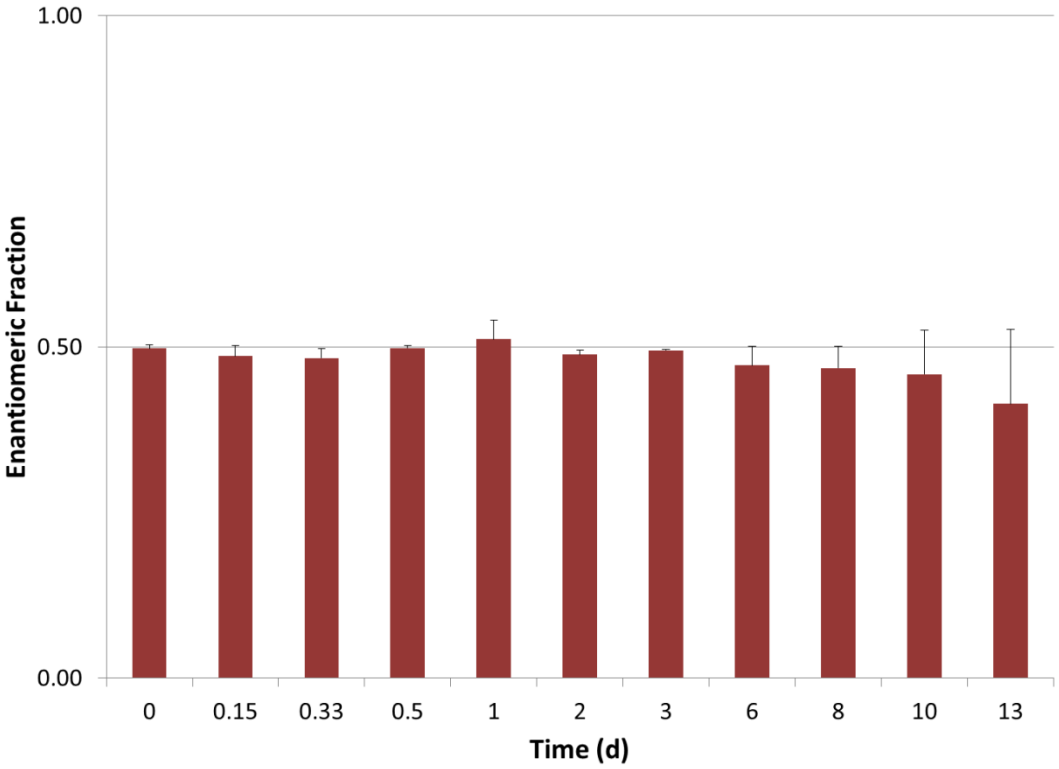


Figure 2.3: *Rac*-malathion change in enantiomeric fractions after incubation in water. Error bars represent standard deviations of triplicates. The change of EF was not significant from t = 0 to t = 13 d (p = 0.38).

controls, racemic malathion did not change in EF (EF = 0.50 ± 0.002). Li et al. (2010) investigated the enantiomerization of malathion and found that it occurred in two aqueous buffers at pH 5.8 and 7.0. Sun et al. (2012) did not provide data for changes in EF for incubation of racemic malathion in water.

To account for the loss of malathion in the controls, the malathion sorbed was corrected for the loss observed in the control for the sorption calculations using the following equation, similar to Oudou and Hansen (2002):

$$\mathrm{M_{min}} = \frac{\mathrm{M_{int}} - [\mathrm{Msol} + (\mathrm{M_{int}} \times \%\mathrm{loss})]}{\mathrm{M_{sorbent}}} \qquad 2.4$$

where M_{min} is the mass of malathion sorbed by the minerals (mg), M_{int} is the initial malathion mass added (mg), M_{sol} is the malathion mass remaining in solution after sorption (mg), %loss is the loss determined for each concentration level for the water controls, and $M_{sorbent}$ is the mass of the sorbent (kg).

Achiral analysis

Sorption equilibration time was chosen as two hours, which did not achieve true equilibrium for the sorption of malathion to the sorbents. As shown in Figure A.1 malathion was not detected in the aqueous solution after eight hours of contact with bentonite. To accurately quantify malathion concentration in solution after sorption,

the two hour time point was chosen to investigate sorption and determine if enantioselective sorption occurred. To quantify malathion in solution after sorption at true equilibrium, either decreasing the sorbent weight or increasing the initial malathion concentration could be attempted. Lower sorbent weights for bentonite were investigated, which are presented below. Increasing the malathion solution concentration was not possible due to the water solubility of malathion at 145 mg/L. The maximum initial malathion solution concentration was at 75% of the water solubility, therefore increasing the concentration was not feasible.

Sorption isotherms for all sorbents were plotted as shown in Figure 2.4 and individual sorption isotherms are in Appendix A (Figures A.6-A.11). Each data point is an average of triplicate measurements, and show acceptable repeatability except for kaolinite (see Figure A.11). Kaolinite has a lower surface area than the other sorbents and in this case is a poor sorbent for malathion; therefore, the erratic sorption data are not surprising. According to the classification by Giles et al. (1974), bentonite, montmorillonite, and calcite exhibit a L-type isotherm, which results when available sorption sites are harder to find because the sorbent surface is covered by sorbate. Although bentonite, montmorillonite, and calcite isotherms exhibit surface coverage, the surfaces do not appear to be near saturation. Diatomaceous earth and seashells, which are both biotic solids, exhibit an S-shaped sorption isotherm, which indicates sorbate-sorbate interactions, which are concentration dependent, resulting in cooperative adsorption (Giles et al., 1974).

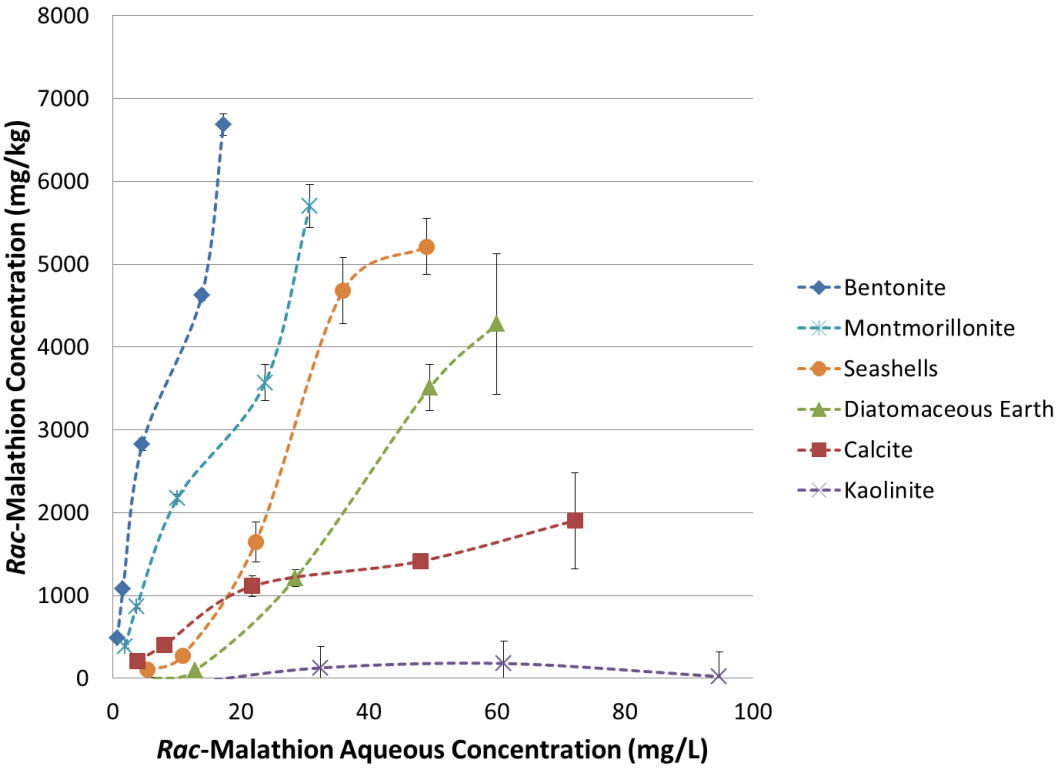


Figure 2.4: *Rac*-malathion sorption isotherms. Dotted lines demonstrate isotherm shapes and do not represent model fits. Error bars represent standard deviations of triplicates. Sorption to kaolinite was negligible.

To quantify sorption processes, the sorption data were fitted to the Freundlich model using the following equation, specifying two hours of equilibration:

$$\log C_S = \log K_{F,2hr} + n_{2hr} \log C_W \qquad 2.5$$

where C_s is the amount of malathion sorbed to the minerals (mg/kg), $K_{F,2hr}$ and n_{2hr} are the Freundlich constant and Freundlich exponent at two hours of equilibration, respectively, and C_w is the amount of malathion remaining in the aqueous phase after sorption (mg/L). Freundlich isotherms are shown in Figure 2.5 and the calculated values in Table 2.1. Coefficients of determination (r^2) indicate that the Freundlich isotherms are linear for all sorbents, except for kaolinite. Since kaolinite exhibited poor sorption capacity for malathion, the erratic sorption data prevented the fitting of the data to the Freundlich model. The value of the Freundlich exponent n_{2hr}, which is an index of the free energies associated with sorption, determines the sorbate concentration dependence of sorption. The n_{2hr} value of bentonite, montmorillonite, and calcite were less than one, indicating that as the concentration of malathion increased, the free energies associated with sorption decreased (Schwarzenbach et al., 2003). The opposite effect was seen with diatomaceous earth and seashells; their n_{2hr} values were greater than one and indicated that as the concentration of malathion increased, the sorption free energies increased as well. It is interesting to note that diatomaceous earth and seashells are biologically derived solids, which could be a factor in sorption.

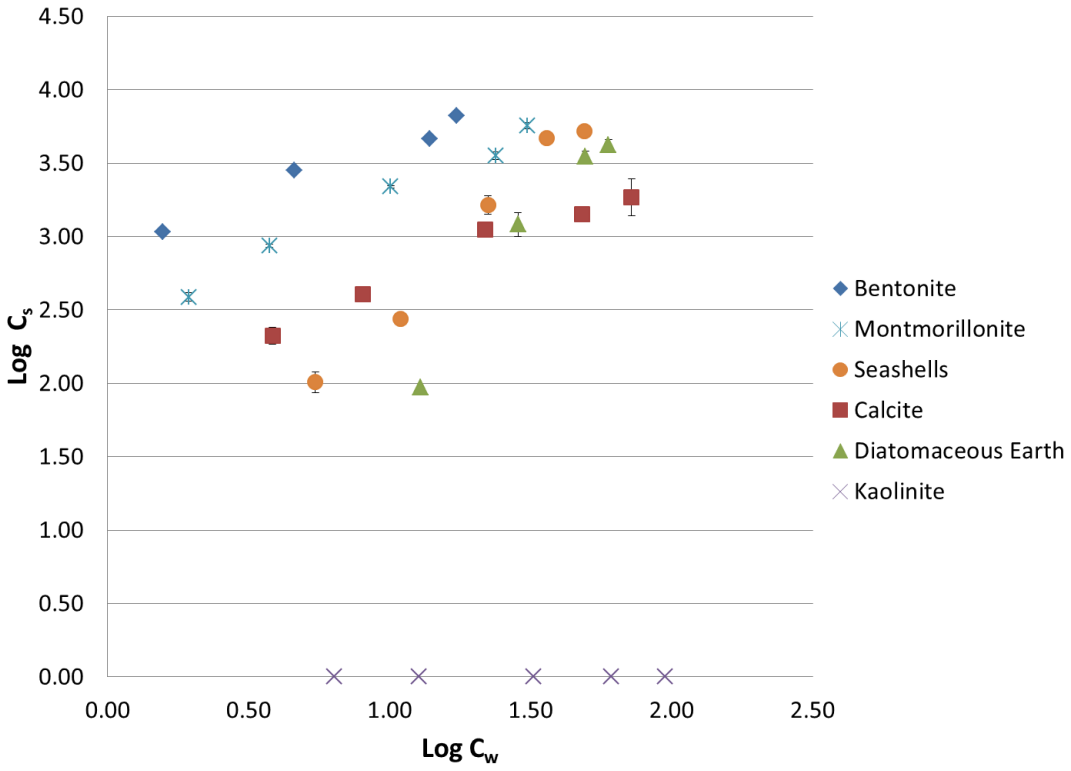


Figure 2.5: *Rac*-malathion Freundlich sorption isotherms. Error bars represent standard deviations of triplicates. C_s = *rac*-malathion sorbed concentration (mg/kg); C_w = *rac*-malathion aqueous concentration (mg/L) after two hours equilibration.

Table 2.1: *Rac*-malathion Freundlich isotherm parameters

Sorbent	n_{2hr}	$K_{F,2hr}$	r^2
Bentonite	0.767 ± 0.034	724 ± 1.07	0.9752
Montmorillonite	0.907 ± 0.038	238 ± 1.10	0.9780
Seashells	1.93 ± 0.09	3.54 ± 1.31	0.9733
Calcite	0.736 ± 0.056	87.3 ± 1.20	0.9311
Diatomaceous Earth	2.48 ± 0.18	0.210 ± 1.87	0.9505

Freundlich exponent n_{2hr} values were statistically different except for bentonite-montmorillonite ($p = 0.07$) and bentonite-calcite ($p = 0.46$) comparisons.

Freundlich constants, which represent the sorption capacity of the sorbent, were significantly different ($p < 0.001$), signifying the different sorption capacities of the sorbents for racemic malathion. Bentonite exhibited the highest sorption capacity, followed by montmorillonite. Bentonite and montmorillonite are smectite clays, which have high surface areas, and a high affinity for malathion. Calcite also exhibited a high sorption capacity for malathion, followed by the much smaller sorption capacities of seashells and diatomaceous earth. Based upon the Freundlich constants, calcite sorption was one order of magnitude smaller than bentonite and montmorillonite, but one order of magnitude larger than diatomaceous earth and seashells. The seashells used in this experiment are assumed to be composed of primarily calcium carbonate, and were expected to have a lower sorption affinity for malathion. Diatomaceous earth is composed of diatoms, which are primarily silicon dioxide, which also would be expected to have a lower sorption capacity for malathion as compared to the smectite clays bentonite and montmorillonite.

The Langmuir model, which assumes there are limited sorption sites, was not used for the sorption of racemic malathion to the selected minerals. The Langmuir model is not applicable in the case of racemic malathion since sorption site capacity was not reached during equilibration according the sorption isotherms (Figure A.1). Importantly, published data of malathion sorption are all presented using the Freundlich

model; therefore, using this model allows appropriate comparison of results. Analyzing the sorption data using the Freundlich model resulted in r^2 near one, indicating the data fit the Freundlich model. The only exception was kaolinite, which exhibited erratic sorption of racemic malathion associated with high error. Kaolinite sorption data were applied to the Langmuir model, and the correlation was improved (r^2 = 0.2743), but the error of the experimental data overshadows the correlation with either Freundlich or Langmuir models (Figure A.12). Therefore, in the case of kaolinite, the Freundlich and Langmuir models may appear to be inadequate to describe the sorption of malathion to kaolinite; however the sorption isotherms for kaolinite indicate that it is an ineffectual sorbent for malathion.

The sorbent:solvent ratio of 50 mg sorbent to 5 mL of solvent was selected based upon a literature review of malathion sorption experiments. Since malathion was not found in aqueous solution after contact with bentonite for approximately 8 hours (see Figure A.1), sorption weight for bentonite was modified to determine the optimal solvent: mass ratio. Sorption isotherms for the original sorbent weight of 50 mg and three other sorbent weights are shown in Figure A.13. All of these isotherms were found to fit the Freundlich model (Figure A.14) with linear coefficients of determination, and the Freundlich parameters are shown in Table 2.2. The n_{2hr} and $K_{F,2hr}$ values were significantly different (p = 0.03; p = 0.02) but n_{2hr} values were still below one and the sorption capacities were on the same order of magnitude. The sorbent weight does not

appear to affect the equilibrium time for malathion sorption to bentonite, therefore 50 mg of sorbent is acceptable for the sorption experiments.

Table 2.2: Bentonite Freundlich isotherm parameters of *rac*-malathion with sorbent weight.

Sorbent	n_{2hr}	$K_{F,2hr}$	r^2
5 mg	0.894 ± 0.075	790.0 ± 1.259	0.9163
12.5 mg	0.790 ± 0.018	1099 ± 1.047	0.9930
25 mg	0.796 ± 0.027	944.3 ± 1.072	0.9851
50 mg	0.767 ± 0.034	724.4 ± 1.072	0.9752

There are only five reports of sorption of malathion to minerals. MacNamara and Toth (1970) examined the sorption of malathion to montmorillonite, kaolinite, and illite using several different exchangeable cations, along with soils and humic acid. Malathion was measured using a colorimetric method and no sorption constants or partition coefficients were calculated; only the concentration of malathion sorbed was presented. Malathion was found to sorb in the decreasing order of montmorillonite, illite, and kaolinite. Kaolinite sorption was at least an order of magnitude lower than sorption to montmorillonite, whereas in my research, no significant sorption of malathion to kaolinite was observed. Mohan et al. (2001) also investigated the sorption of malathion to montmorillonite and kaolinite using different exchangeable cations using a spectrophotometric method. The Freundlich model was used to describe the

sorption data, however no calculated Freundlich parameters or linear regressions were provided. Since no data were provided, only Freundlich isotherm plots, comparison is difficult. However, the plots appear to be linear for both montmorillonite and kaolinite in contrast to the non-linear data for kaolinite presented in my research. Gonzalez-Pradas et al. (1993) investigated the sorption of malathion to bentonite with varied exchangeable cations and found sorption to fit the Freundlich model. Analysis was performed using a spectrophotometric method with an equilibration time of 24 hours and as shown in Table 2.3 their *n* values were lower than reported here and the K_F values are lower as well, but within the same order of magnitude.

Table 2.3: *Rac*-malathion Freundlich isotherm parameters comparison

Sorbent	Current	Reference A	Reference B
Bentonite	n_{2hr} = 0.767 $K_{F,2hr}$ = 724	*n* = 0.42-0.45 K_F =150-380	*n* = 1.124 K_F =367
Montmorillonite	n_{2hr} = 0.907 $K_{F,2hr}$ = 238	N/A	*n* = 1.050 K_F =562
Kaolinite	N/A	N/A	*n* = 3 K_F = 1077

N/A = Not applicable. Constants not determined.
Reference A = Gonzalez-Pradas et al. (1993)
Reference B = Pal and Vanjara (2001)

Pal and Vanjara (2001) investigated sorption of malathion to bentonite, montmorillonite, and kaolinite using a spectrophotometric method and found that the sorption data fit the Freundlich model with 24 hours equilibration time. As indicated in Table 2.3, the K_F of bentonite and montmorillonite sorption of malathion were on the same order of magnitude, as presented here, and the *n* value was slightly higher with *n* > 1. However, the kaolinite data are not comparable, and according to the calculated K_F, malathion was sorbed to a greater extent by kaolinite, followed by montmorillonite and bentonite; this is the opposite observed in my research. Their results are surprising because kaolinite has a lower surface area and cation exchange capacity. When the three clays were treated with tetradecyltrimethyl ammonium bromide (TTAB), bentonite-TTA resulted in the highest K_F, followed by equivalent K_F for both kaolinite-TTA and montmorillonite-TTA. In addition, the adsorption (removal) efficiency of the clays for malathion was calculated using the following equation:

$$R = \frac{(C_0 - Ce) \times 100\%}{C_0} \qquad 2.6$$

where C_0 is the initial aqueous concentration of malathion, C_e is the concentration of malathion in aqueous solution after equilibration, and R is expressed in percentage. The results presented indicate that kaolinite has the lowest removal efficiency of malathion followed by bentonite and then montmorillonite. Based upon removal efficiency, the

order of sorption would decrease in the order of montmorillonite, bentonite, and kaolinite, which is more aligned with my research. The removal efficiencies and the Freundlich constants present conflicting results about the sorption of malathion to the three clays.

Donia et al. (2011) measured the sorption of malathion to bentonite, kaolinite and thermally treated kaolinite at 400 and 600^{o}C using spectrophotometry analysis at 195 nm. Significant sorption of malathion was found with all bentonite and kaolinite samples, and sorption increased as a function of temperature and sorbent weight. The higher temperatures were hypothesized to increase the hydrophobicity of kaolinite by dehydration and hydroxylation and subsequently increased the sorption of partially hydrophobic malathion. The significant sorption of malathion to kaolinite is in contrast to my results where sorption to kaolinite was not apparent. The differences in the results may be due to the use of a nonspecific spectrophotometric method at 195 nm, which could result in matrix interferences causing abnormally high absorbances. It is important to note that the kaolinite used in my research was a source clay from the Clay Minerals Society which has been homogenized and thoroughly characterized. On the contrary, the kaolinite materials used in the reports mentioned previously are from commercial sources such as Merck or Pharma (Donia et al., 2011; Gonzalez-Pradas et al., 2003; Pal and Vanjara, 2001). The differences in kaolinite sources may account for the differences observed with malathion sorption to kaolinite along with differences in analytical detection of malathion.

Chiral Analysis

Results for the chiral analysis of the sorption of racemic malathion to the selected minerals are shown in Figure 2.6. All enantiomeric fractions were measured in triplicate with very low error. All enantiomeric fractions were racemic for all sorbents and malathion concentrations. Racemic malathion controls without sorbent or with β-cyclodextrin also remained racemic with EFs = 0.5 (data not shown). Controls with the chiral stationary phase cellulose tris-(4-methylbenzoate) were inconclusive due to interference with the *S*-(-)-malathion peak. For this research, malathion aqueous controls containing no sorbent were racemic after equilibration time, thus eliminating possible bias of the chiral sorption data of malathion. Nonenantioselective sorption of malathion to the selected minerals is somewhat surprising given the chirality of the sorbents used. Based upon the presented results, the current consensus, at least for malathion, is correct; sorption as a physical process is a nonenantioselective process in the environment.

Conclusions

Sorption of racemic malathion to minerals was investigated by achiral and chiral analysis. Racemic malathion was found to sorb to bentonite, calcite, diatomaceous earth, montmorillonite, and seashells. However, sorption to kaolinite was negligible, indicating kaolinite is an ineffectual sorbent for malathion. Sorption by the minerals, with the exception of kaolinite, was found to adhere to the Freundlich isotherm model,

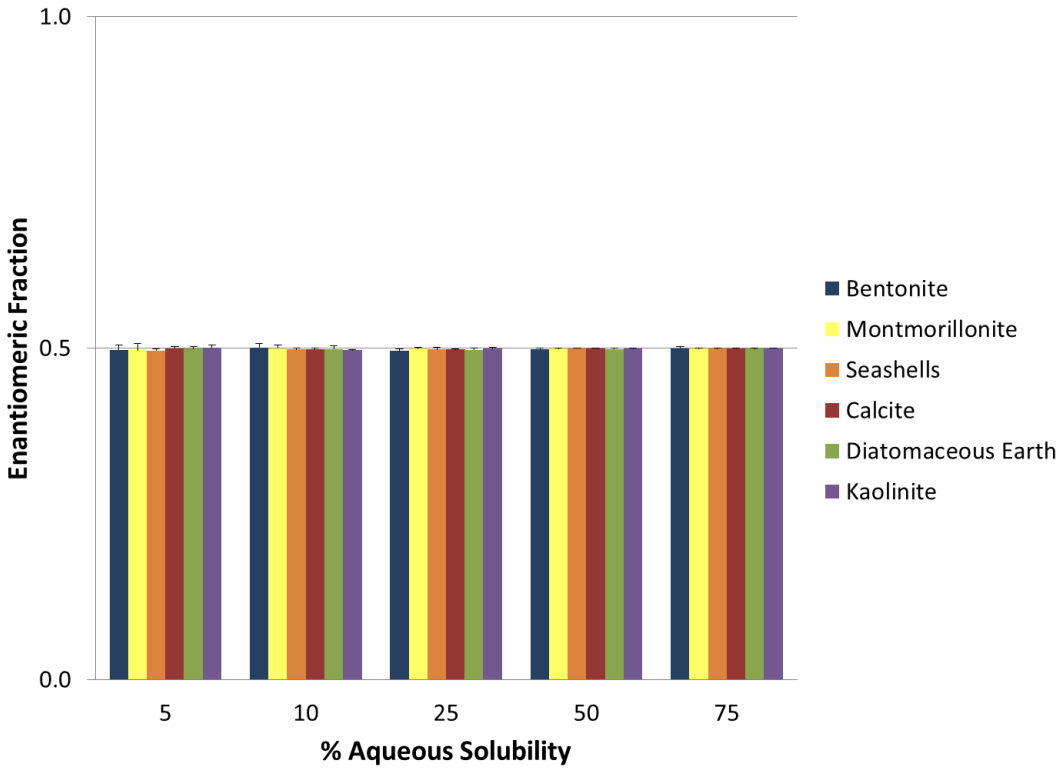


Figure 2.6: Enantiomeric fractions of *rac*-malathion sorption. Error bars represent standard deviations of triplicates.

with r^2 = 0.9311-0.9780. The Freundlich exponent n_{2hr} values were less than one for bentonite, calcite, and montmorillonite, while for the biotic solids diatomaceous earth and seashells, they were greater than one. Calculated Freundlich constants $K_{F.2hr}$ indicated sorption of malathion in the decreasing order of montmorillonite, bentonite, calcite, seashells, and diatomaceous earth. Chiral investigation of the sorption of racemic malathion showed that sorption for all sorbents was nonenantioselective as indicated by racemic enantiomeric fractions. This study presents the first sorption results for malathion using chiral HPLC analysis and is only one of the few records of sorption of malathion in the literature. Importantly, this study provides more accurate quantitative sorption data by using achiral HPLC versus nonspecific spectrophotometric methods that can result in biased data due to matrix interferences. Malathion has been proven to sorb readily to minerals, therefore, inferring sorption to more complex matrices such as soils and sediments. Malathion is only slightly water soluble; therefore, sorption would be expected. This can reduce its bioavailability, and in turn, prevent microbial degradation, increasing its persistence in the environment. Sorption could also prevent the oxidation of malathion to the more toxic metabolite malaoxon. However, if soils contain high amounts of kaolinite, leaching of malathion may occur due to the observed negligible sorption of malathion by kaolinite. As a registered USEPA chiral pesticide used in high volumes, additional research on the environmental fate processes of malathion is required to understand the implications of its widespread applications.

References

Asthagiri, A.; Hazen, R. M. An ab inito study of adsorption of alanine on the chiral calcite surface. *Molecular Simulation* **2007**, *33*, 343-351.

Berthod, A. Chiral recognition mechanisms. *Analytical Chemistry* **2006**, *78*, 2093-2099.

Bondarenko, S.; Gan, J. Degradation and sorption of selected organophosphate and carbamate insecticides in urban stream sediments. *Environmental Toxicology and Chemistry* **2004**, *23*, 1809-1814.

Bondy, S. C.; Harrington, M. E. L-Amino acids and D-glucose bind stereoselectively to a colloidal clay. *Science* **1979**, *203*, 1243-1244.

Bonner, W. A.; Kavasmaneck, P. R.; Martin, F. S. Flores, J. T. Asymmetric adsorption of alanine by quartz. *Science* **1974**, *186*, 143-144.

Donia, A. M.; Atia, A. A.; Hussein, R. A.; Rashad, R. T. Efficient adsorption of malathion from different media using thermally treated kaolinite. *Desalination and Water Treatment* **2011**, *30*, 178-185.

Ellington, J. J.; Evans, J. J.; Prickett, K. B.; Champion Jr., W. L. High performance liquid chromatographic separation of enantiomers of organophosphorus pesticides on polysaccharide chiral stationary phases. *Journal of Chromatography A* **2001**, *928*, 145-154.

Garrison, A. W. Probing the enantioselectivity of chiral pesticides. *Environmental Science and Technology* **2006**, *40*, 16-23.

Gellman, A. J. Chiral surfaces: Accomplishments and challenges. *ACS Nano* **2010**, *4*, 5-10.

Giles, C. H.; Smith, D.; Huitson, A. A general treatment and classification of the solute adsorption isotherm. *Journal of Colloid and Interface Science* **1974**, *47*, 755-765.

Gonzalez-Pradas, E.; Villafranca-Sanchez, M.; Fernandez Perez, M.; Socias Viciana, M. Adsorption of malathion from aqueous solution on homoionic bentonite samples. *Agrochimica* **1993**, *37*, 104-110.

Han, J. W.; Sholl, D. S. Enantiospecific adsorption of amino acids on hydroxylated quartz (0001). *Langmuir* **2009**, *25*, 10737-10745.

Han, J.W.; Sholl, D. S. Enantiospecific adsorption of amino acids on hydroxylated quartz (10$\bar{1}$0). *Physical Chemistry Chemical Physics* **2010**, *12*, 8024-8032.

Hazen, R. M.; Filley, T. R.; Goodfriend, G. A. Selective adsorption of L-and D-amino acids on calcite: Implications for biochemical homochirality. *Proceedings of the National Academy of Sciences* **2001**, *98*, 5487-5490.

Hazen, R. M.; Sholl, D. S. Chiral selection on inorganic crystalline surfaces. *Nature Materials* **2003**, *2*, 367-374.

Hegeman, W. J. M.; Laane, R. W. P. M. Enantiomeric enrichment of chiral pesticides in the environment. *Reviews of Environmental Contamination and Toxicology* **2002**, *173*, 85-116.

Horvath, J. D.; Gellman, A. J. Naturally chiral surfaces. *Topics in Catalysis* **2003**, *25*, 9-15.

Julg, A.; Ozias, Y. Asymmetric adsorption of ethyliminium cation on kaolinite and L-homochirality of amino acids in proteins. *Journal of Molecular Structure (Theochem)* **1988**, *179*, 17-25.

Kohler, H. P., Angst, W.; Giger, W. Kanz, C.; Muller, S.; Suter, M. Environmental fate of chiral pollutants – the necessity of considering stereochemistry. *Chimia* **1997**, *51*, 947-951.

Kulluru, P.P.; Das, B. S.; Panda, R, K. Evaluation of sorption and leaching potential of malathion and atrazine in agricultural soils of India. *International Journal of Environmental Research* **2010**, *4*, 75-90.

Lewis, D, L.; Garrison, A. W.; Wommack, K. E.; Whittemore, A.; Steudler, P.; Mellilo, J. Influence of environmental changes on degradation of chiral pollutants in soils. *Nature* **1999**, *401*, 898-901.

MacNamara, G.; Toth, S. J. Adsorption of linuron and malathion by soils and clay minerals. *Soil Science* **1970**, *109*, 234-239.

Matallo, M. Romero, E. Sanchez-Rasero, F. Adsorption of mecoprop and dichlorprop on calcerous and organic matter amended soils. *Journal of Environmental Science and Health, Part B: Pesticides Food Contaminants and Agricultural Wastes* **1998**, *33*, 51-66.

Mohan, K. R.; Ramesh, A.; Seshaiah, K. Studies on adsorption of selected organophosphorus pesticides by clays. *Indian Journal of Chemical Technology* **2001**, *8*, 278-280.

Muller, M. D.; Buser, H. –R. Conversion reactions of various phenoxyalkanoic herbicides in soil. 1. Enantiomerization and enantioselective degradation of the chiral 2-phenoxypropionic acid herbicides. *Environmental Science and Technology* **1997**, *31*, 1953-1959.

Oravec, M.; Simek, Z.; Holoubek, I. The effect of humic acid and ash on enantiomeric fraction change of chiral pollutants. *Colloids and Surfaces A: Physiochemical Engineering Aspects* **2010**, *359*, 60-65.

Oudou, H. C.; Hansen, H. C. B. Sorption of lambda-cyhalothrin, cypermethrin, deltamethrin and fenvalerate to quartz, corundum, kaolinite and montmorillonite. *Chemosphere* **2002**, *49*, 1285-1294.

Pal, O. R.; Vanjara, A. K. Removal of malathion and butachlor from aqueous solution by clays and organoclays. *Separation and Purification Technology* **2001**, *24*, 167-172.

Polec, I.; Cieslak, L.; Sledzinski, B.; Ksycinska, H. Simple syntheses of malathion and malaoxon enantiomers, and isomalathion diastereoisomers: Toxicity-configuration relationship. *Pesticide Science* **1998**, *53*, 165-171.

Sanchez Camazano, M.; Sanchez Martin, M. J. Factors influencing interactions of organophosphorus pesticides with montmorillonite. *Geoderma* **1983**, *29*, 107-118.

Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M. *Environmental Organic Chemistry*. **2003**, 2nd Edition. John Wiley and Sons, Inc.

Siffert, B.; Naidja, A. Stereoselectivity of montmorillonite in the adsorption and deamination of some amino acids. *Clay Minerals* **1992**, *27*, 109-118.

Sujatha, C. H.; Chacko, J. Malathion sorption by sediments from a tropical estuary. *Chemosphere* **1991**, *23*, 167-180.

Sun, M.; Liu, D.; Zhou, G.; Li, J.; Qui, X.; Zhou, Z.; Wang, P. Enantioselective degradation and chiral stability of malathion in environmental samples. *Journal of Agricultural and Food Chemistry* **2012**, *60*, 372-379.

Wedyan, M.; Preston, M. R. Isomer-selective adsorption of amino acids by components of natural sediments. *Environmental Science and Technology* **2005**, *39*, 2115-2119.

Wolfe, N. L.; Zepp, R. G.; Baughman, G. L.; Gordon, J. A. Kinetic investigation of malathion degradation in water. *The Bulletin of Environmental Contamination and Toxicology* **1975**, *13*, 707-713.

Wong, C. S. Environmental fate processes and biochemical transformations of chiral emerging organic pollutants. *Annuals of Bioanalytical Chemistry* **2006**, *386*, 544-558.

Zhang, A.; Xie, X.; Ye, J.; Lin, C.; Hu, X. Stereoselective toxicity of malathion and its metabolites, malaoxon and isomalathion. *Environmental Chemistry Letters* **2011**, *9*, 369-373.

Zipper, C.; Suter, M, J.; Haderlein, S. B.; Gruhl, M.; Kohler, H. P. Changes in the enantiomeric ratio of (*R*)- to (*S*)-mecoprop indicate in situ biodegradation of this chiral herbicide in a polluted aquifer. *Environmental Science and Technology* **1998**, *32*, 2070-2076.

CHAPTER 3

SORPTION AND ENANTIOMERIZATION OF MALATHION ENANTIOMERS BY MINERAL SURFACES

Abstract

Sorption and enantiomerization of *R*-(*+*)- and *S*-(-)-malathion were investigated using achiral and chiral analysis and compared to the racemate results. Malathion enantiomers were stable in the aprotic HPLC eluent, but enantiomerized in the presence of water. The enantiomers also underwent first-order degradation, with *R*-(*+*)-malathion degrading the fastest, followed by the racemate and the *S*-(-)-enantiomer. Sorption of malathion enantiomers for two hours resulted in enantiomerization to racemic for bentonite, calcite, diatomaceous earth, montmorillonite, and seashells, independent of the starting enantiomer. Addition of the malathion enantiomers to all sorbents followed by immediate analysis resulted in enantiomerization to racemic of *R*-(*+*)-malathion and partial enantiomerization of *S*-(-)-malathion. Aqueous controls containing β-cyclodextrin also indicated significant, but not complete enantiomerization to racemic. Difference in sorption curves was observed for the enantiomers and the racemate indicating the possibility of separate sorption processes. Sorption was found to fit the Freundlich model, with $n_{2hr} < 1$; sorption capacity decreased in the order of bentonite, montmorillonite, and calcite. Comparison of the malathion racemate and enantiomers indicated a significant difference in n_{2hr} for bentonite, but not for calcite

and montmorillonite. Freundlich constants ($K_{F,2hr}$) were not significantly different indicating that the sorbents have the same capacity for racemic, *R*-(*+*)-, and *S*-(-)-malathion.

Introduction

Chiral sorption is a phenomenon that is currently driving homochirality research, but is often overlooked in regards to environmental contaminants. Vital to origin-of-life theories, homochirality is the existence of only one enantiomer of an optically active biological compound; for example, L-amino acids and D-sugars. For chiral sorption to occur, the sorbate and sorbent must be asymmetric, i.e. chiral. Theoretically, at least three interaction points must occur between the chiral molecule and the chiral surface for selective sorption to occur (Berthod, 2006; Hazen and Sholl, 2003). In fact, chiral analyses are achieved using the concepts of chiral sorption, wherein enantiomers of a chiral compound are separated and quantified by enantiospecific reactions with chiral stationary phases.

In the environment, there are abundant possible chiral surfaces such as quartz, which displays left- and right-handed helix structures, and kaolinite, with asymmetric triclinic crystals (Horvath and Gellman, 2003). Calcite is achiral, but can display chiral crystal faces; montmorillonite is achiral as well, but can display chirality in the stacking of its structural units (Hazen and Sholl, 2003; Julg and Ozias, 1988; Siffert and Naidja, 1992). Soils and sediments, which contain organic matter, can also display chirality and

possibly induce chiral sorption. Enantioselective amino acid sorption has been observed by modeling or laboratory experiments with minerals (Asthagiri and Hazen, 2007; Bondy and Harrington, 1979; Bonner et al., 1974; Han and Sholl, 2009; Han and Sholl, 2010; Hazen et al., 2001; Siffert and Naida, 1992) and with sediment (Wedyan and Preston, 2005). However, there are only a few reports of possible chiral sorption of pesticides to environmental surfaces (Matallo et al., 1998; Oravec et al., 2010; Zipper et al., 1998).

Isomerization, including racemization and enantiomerization, of chiral pesticides is also a concern that is often overlooked in environmental research. Racemization is the macroscopic process that occurs when one enantiomer irreversibly transforms to the other enantiomer whereas enantiomerization is the microscopic process of the reversible interconversion of enantiomers (Reist et al., 1995; Wolf, 2008). Isomerization of chiral pesticides is problematic in the application of enantiopure pesticides. Enantioselective toxicity research has proven that the biological activity of some chiral pesticides resides in only one enantiomer, such as the case with metalaxyl, where the fungicidal activity is due to the *R*-enantiomer (Buser et al., 2002). If enantiomerization or racemization occurs with a pesticide that is applied as one enantiomer, then the effectiveness of the pesticide is reduced if the non-active enantiomer is formed once introduced to the environment. Therefore, isomerization of chiral pesticides should be considered when investigating sorption processes. To date, there are no reports of chiral pesticide isomerization during sorption, but there are reports of isomerization during degradation studies. Enantiomerization of the chiral herbicides mecoprop and

dichlorprop has been observed in soil degradation studies (Messina and Sinibaldi, 2007; Muller and Buser, 1997; Romero et al., 2001) and the pyrethroids fenpropathrin and fenvalerate enantiomerized in soils as well (Li et al., 2009). Enantiomerization and racemization can also occur in abiotic solvents as shown with the pyrethroids cypermethrin and fenpropathrin and the organophosphorus pesticides malathion and phenthoate (Li et al., 2010; Nillos et al., 2009; Qin and Gan, 2007). Enantiomerization and racemization are commonly used interchangeably, but in this dissertation, enantiomerization is used to define interconversion of enantiomers resulting in a mixture that may or may not be racemic.

Organophosphorus pesticides are insecticides in wide use today due to the phasing out of organochlorine pesticides. Malathion is a chiral organophosphate pesticide used in the United States as a broad-spectrum insecticide, containing one chiral carbon, consisting of one enantiomer pair. Malathion is sold exclusively as a racemate although enantioselective toxicity research indicates that *R*-(*+*)-malathion is more toxic than *S*-(-)-malathion to rats, anthropods, and *Daphnia magna* (Polec et al., 1998; Zhang et al., 2011). Although almost 15 million pounds of malathion was used annually in 2006, relatively little research as to the environmental fate of malathion has been conducted (EPA, 2006). Malathion sorption to minerals, soils, and sediments has been reported (Bondarenko and Gan, 2004; Donia et al., 2011; Gonzalez-Pradas et al., 1993; Kulluru et al., 2010; MacNamara and Toth, 1970; Mohan et al., 2001; Pal and Vanjara, 2001; Sanchez-Camazano and Sanchez Martin, 1983; Sujatha and Chacko, 1991;

Uchimiya et al., 2012) but only one report of enantioselective degradation has been published (Sun et al., 2012).

Chapter 2 of this dissertation presented the first investigation of malathion sorption using chiral analysis to investigate the possibility of enantioselective sorption to minerals. The purpose of this chapter was to investigate the sorption of individual malathion enantiomers to mineral surfaces to test the hypothesis that the enantiomers show significant differences in sorption and to consider the effects of sorption on enantiomerization. Sorption investigation of individual enantiomers instead of only the racemate would eliminate competitive sorption between the two enantiomers and allow direct investigation of single enantiomer sorption. Using single enantiomers instead of only racemates would also elucidate the possibility of enantiomerization when the enantiomers are either in aqueous solution or in contact with the mineral surface, which would not be obvious in investigating only sorption of the racemic pesticide.

Materials and Methods

Chemicals and materials

Racemic malathion (> 97%), malathion monocarboxylic acid (technical grade), malaoxon (94.3%), and isomalathion (100 µg/mL in hexane) were obtained from Chem Service (West Chester, PA). *R*-(*+*)-malathion and *S*-(-)-malathion were separated by chiral HPLC and collected from the outlet of the UV detector in a mobile phase

consisting of 90:10 hexane:reagent alcohol. Enantiomers were stored in acetone and enantiomeric purities determined by chiral HPLC were ≥ 94% for *R*-(*+*)-malathion and ≥ 95% for *S*-(-)-malathion. Reagent grade acetone and HPLC grade acetonitrile and hexanes were obtained from Fisher Chemicals (Fair Lawn, NJ). Reagent alcohol, which is a mixture of approximately 90:5:5 ethanol: methanol: isopropanol was obtained from Richard-Allan Scientific (Kalamazoo, MI). Deionized water (> 18 MΩ) was used for all experiments. Montmorillonite (SWy-2) was obtained from the Clay Minerals Society (West Lafayette, IN). Calcite crystals, ranging from 0.2-0.5 cm, were obtained from Alfa Aesar (Ward Hill, MA) and bentonite and diatomaceous earth were obtained from Spectrum Chemicals (New Brunswick, NJ). Seashells collected from various locations along the US east coast were crushed to coarse particles before use in sorption experiments. Bentonite, montmorillonite, kaolinite, calcite, and diatomaceous earth were used as received, with no additional treatments. The chiral stationary phase, cellulose tris-(4-methylbenzoate) was donated by Chiral Technologies (West Chester, PA) and β-cyclodextrin was obtained from Sigma Aldrich (St. Louis, MO).

Malathion stability experiments

R-(*+*)- and *S*-(-)-malathion enantiomers were separated by chiral HPLC and stored in hexane: reagent alcohol (90:10) at 4^{o}C, protected from light. Aliquots were sampled daily and tested by chiral HPLC to determine the enantiomeric fraction (EF), as determined by the following equation:

$$\mathrm{EF} = \frac{\text{R-(+)}}{\text{R-(+)} + \text{S-(-)}} \qquad 3.1$$

where *R*-(*+*) and *S*-(-) represent the respective peak areas of the malathion enantiomers.

To accurately analyze small changes in concentration or EFs, individual malathion enantiomer aqueous solutions of approximately 10 mg/L were prepared by adding an aliquot of either *R*-(*+*)- or *S*(-)-malathion in acetone to 50 mL glass centrifuge tubes, evaporating to dryness with nitrogen, and reconstituting in 40 mL of water. The experiment was conducted in triplicate, and the centrifuge tubes were placed on a wrist action shaker, protected from light. A 2 mL aliquot was removed for both achiral and chiral analysis of the malathion enantiomers.

Sorption experiments

Enantiospecific sorption of malathion to bentonite, calcite, and montmorillonite

Separate batch sorption experiments were conducted with *R*-(*+*)- or *S*-(-)-malathion at concentration levels ranging from 4-122 mg/L. Malathion concentration ranges were determined from a comprehensive literature review indicating that malathion sorption experiments had initial aqueous concentrations that ranged from 4-750 mg/L, therefore malathion enantiomer concentrations were selected to fall within this range (Donia et al., 2011; Gonzalez-Pradas, et al., 1993; MacNamara and Toth, 1970; Mohan et al., 2001). To Pyrex test tubes, 50 mg of sorbent and 5 mL of aqueous malathion enantiomer solutions were added. Control samples using either water, β-

cyclodextrin (1 g sorbent weight due to water solubility of β-cyclodextrin), or chiral stationary phase cellulose tris-(4-methylbenzoate) were prepared as well. All experiments were conducted in triplicate. Equilibration time of two hours was chosen based upon the time study conducted for racemic malathion detailed in Chapter 2 of this dissertation. Sorption samples were vortexed briefly, placed on a wrist action shaker for two hours, protected from light, and the phases were separated by centrifugation at 1000 rpm for 15 min. The concentrations of *R*-(*+*)- and *S*-(-)-malathion were determined by achiral HPLC analysis and EFs were determined by chiral HPLC analysis.

Enantiomerization experiments

Separate batch experiments using aqueous solutions of *R*-(*+*)- or *S*-(-)-malathion at approximately 12 mg/L were conducted in triplicate using either 5, 12.5, of 25 mg of bentonite as the sorbent. Samples using 50 mg of calcite, diatomaceous earth, and seashells were also prepared in triplicate. Five mL of malathion enantiomer aqueous solutions were added to the solids in Pyrex test tubes. Samples were vortexed for 5 sec and immediately centrifuged at 1000 rpm for 15 min. An aliquot was removed and tested by chiral HPLC to determine the EFs.

Achiral HPLC analysis

Analysis was performed on a Waters Alliance 2690 Separation model HPLC equipped with a 2996 PDA detector and Empower Pro chromatography software (Waters Corporation, Milford, MA). A Zorbax SB-C18 3.5 µm rapid resolution column, 4.6 x 100 mm (Agilent Technologies, Wilmington, DE) was used and the mobile phase was isocratic 50:50 acetonitrile:water at 1.5 mL/min. Analysis time was 10 min, with a 50 µL injection volume and ambient column temperature. Malathion absorbance was measured at 210 nm and calculated using a linear regression plot through the origin of six concentration levels ranging from 0.24-160 mg/L malathion (r^2 = 0.9995-0.9999) with LOD = 0.028 mg/L. For multi-phase samples, an aliquot of the aqueous layer was diluted 1:1 with acetonitrile, filtered through a 0.45 µm hydrophobic PFTE syringe filter into a HPLC autosampler vial, and analyzed. Malathion extraction recoveries were > 96%. Achiral analysis was used for quantitative analysis of sorption, considered as a loss of malathion from solution, with no consideration of chirality.

Chiral HPLC analysis

Analysis was adapted from Ellington et al. (2001) using a 4.6 mm x 250 mm Chiralcel® OJ® packed with cellulose tris-(4-methylbenzoate) coated on a 10 µm silica gel substrate (Chiral Technologies, West Chester, PA). The mobile phase consisted of 90:10 hexane: reagent alcohol at 0.9 mL/min with an analysis time of 25 min on a Perkin Elmer Series 200 HPLC system equipped with UV detection and TotalChrom 6.3.2

chromatography software (Perkin Elmer, Waltham, MA). The column temperature was 20^{o}C and the Peltier autosampler was 10^{o}C, with 100 uL injection volume. Malathion absorbance was measured at 210 nm, and the malathion enantiomers were baseline resolved: *R*-(*+*)-malathion eluted first, at approximately 11.3 min followed by *S*-(-)-malathion at 13.7 min. LOD for both *R*-(*+*)- and *S*-(-)-malathion was 0.9 mg/L. For chiral analysis, an aliquot of the aqueous layer of the samples was extracted with 800 µL hexane and transferred to an HPLC vial for analysis. Recoveries of *R*-(*+*)- and *S*-(-)-malathion demonstrated no change in EF, and racemic malathion standard was analyzed throughout analysis to verify the method was accurate. Chiral analysis was used to calculate EFs to determine if enantiomerization or enantioselective sorption occurred for malathion.

Statistical analysis

Statistical analysis for rate constants and Freundlich isotherm parameters was performed with R statistical software (R Foundation for Statistical Computing, 2012) using regression analysis with a qualitative predictor for the enantiomeric form of malathion by comparing the slopes (rate constants or Freundlich exponent *n*) or intercepts (Freundlich capacity factor K_F) with $\alpha = 0.05$. Statistical analysis for EFs was performed using single factor ANOVA in Microsoft Excel (Microsoft Office 2010), using $\alpha = 0.05$. P values are reported throughout the results and discussion and $p < 0.05$ is determined to be statistically significant whereas $p > 0.05$ is not statistically significant.

EF results are presented with standard deviations whereas the rate constant, half-life, and Freundlich isotherm parameters are presented with standard error for linear regression analysis.

Results and Discussion

Malathion stability

Chiral stability

Incubation of *R*-(*+*)- and *S*-(-)- malathion in the chiral HPLC eluent, 90:10 hexane: reagent alcohol, after 13 days at 4^{o}C resulted in no significant change in EF. *R*-(*+*)-malathion EF was equal to 1.00 at t = 0 d and remained the same at t = 13 d. *S*-(-)-malathion EF was equal to 0.0007 at t = 0 d and increased to EF = 0.0008 at t = 13 d, but the change was not significant. However, incubation of the malathion enantiomers in water after 13 days resulted in enantiomerization. Figure 3.1 shows the plot of malathion EF versus time for *R*-(*+*)- and *S*-(-)-malathion and racemic malathion. Over time, *R*-(*+*)-malathion EF decreased and *S*-(-)- malathion increased, moving towards a racemic EF = 0.5. Sun et al. (2012) also observed enantiomerization of malathion in environmental water samples and Li et al. (2010) observed malathion enantiomerization in aqueous buffered solutions at pH 5.8 and 7.0.

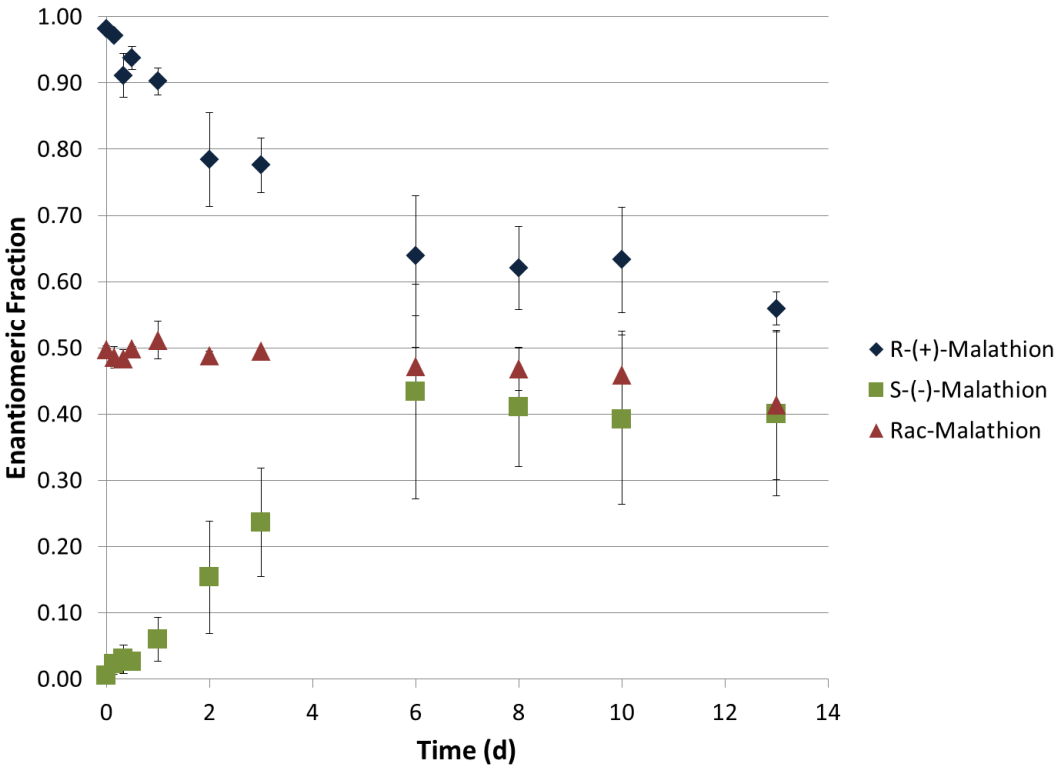


Figure 3.1: Change in enantiomeric fractions for malathion after incubation in water. Error bars represent standard deviations of triplicates.

Degradation

Degradation of malathion enantiomers occurred in water after 13 days based upon achiral analysis, as indicated by Figure 3.2. The degradation followed first order kinetics and the rate constant k and the half-life $t_{1/2}$ were calculated from a regression plot of ln (C_t) versus time (Figure B.1). The coefficient of determinations were r^2 = 0.9157 for *R*-(*+*)-malathion and lower for *S*-(-)-malathion at r^2 = 0.6976. The lower r^2 value for *S*-(-)-malathion can be explained by the experiment being conducted in triplicate, i.e. three separate test tubes, which resulted in different kinetics for each tube. One test tube for *S*-(-)-malathion had a consistently lower concentration in comparison to the other two tubes and resulted in a higher error, resulting in a lower r^2 than *R*-(*+*)-malathion. Rate constants and half-lives were k = 0.134 (± 0.007) d^{-1}, $t_{1/2}$ = 5.17 (± 0.28) d for *R*-(*+*)-malathion and k = 0.087 (± 0.010) d^{-1}, $t_{1/2}$ = 8.00 (± 0.95) d for *S*-(-)-malathion. From these results, the *R*-(*+*)-enantiomer degraded faster than the *S*-(-)-enantiomer with significant differences in k (p = 0.005). In comparison to the malathion racemate (k = 0.107 ± 0.016 d^{-1}; $t_{1/2}$ = 6.48 ± 0.97 d), there was a significant difference in k for *rac*- and *S*-(-)-malathion (p = 0.019) but not for *rac*- and *R*-(*+*)-malathion (p = 0.097). Sun et al. (2012) also observed malathion degradation in water, using environmental samples including rain, canal, reservoir, and ground water. Their reported rate constants ranged from 0.2114—0.8305 d^{-1} and $t_{1/2}$ = 0.77—3.94 d for malathion racemate and enantiomers, indicating a faster degradation rate than observed in this research and more rapid degradation of *S*-(-)-malathion. Higher rate constants and

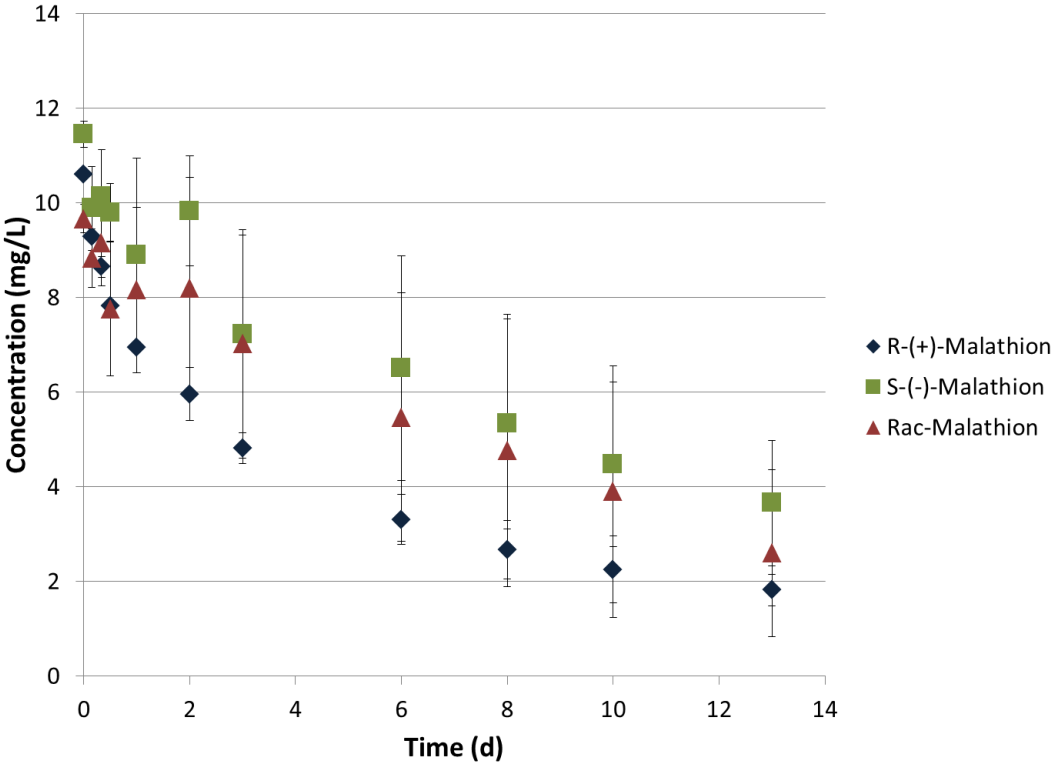


Figure 3.2: Degradation of malathion in water. Error bars represent standard deviations of triplicates.

shorter half-lives could be attributed to the use of environmental samples in Sun et al. versus the deionized water in this research. Environmental samples would be more likely to contain microorganisms causing biotic degradation.

Controls

After two hours, controls containing only aqueous *R*-(*+*)- or *S*-(-)-malathion showed significant enantiomerization at two concentration levels for *R*-(*+*)-malathion and three concentration levels for *S*-(-)-malathion as shown in Figure B.2. As with the kinetic experiment, EFs moved toward racemic values for both enantiomers. Controls containing aqueous enantiomer, and β-cyclodextrin showed enantiomerization, by moving toward EF = 0.5. *R*-(*+*)-malathion EF decreased from EF = 0.99 (± 0.0003) to EF = 0.85 (± 0.02) and *S*-(-)-malathion EF increased from EF = 0.003 (± 0.0007) to EF = 0.06 (± 0.01). Controls with the chiral stationary phase cellulose tris-(4-methylbenzoate) were inconclusive due to interference with the *S*-(-)-malathion peak.

Degradation also occurred in the water-only controls at several concentration levels as shown in Figures B.3. As with the malathion racemate, an unknown peak was present in some of the achiral HPLC chromatograms, which could be a breakdown product of malathion in water. Retention time comparison of solutions of malathion monocarboxylic acid, isomalathion, and malaoxon confirmed that the unknown peak was not one of these known degradation products (Figure B.4; Wolfe et al., 1975). However, malathion is known to produce other degradation products in water (shown

in Figure A.5) that were not included in the analysis for this research due to the availability of reference standards (Wolfe et al., 1975). This unknown peak also appeared in some of the chromatograms after sorption of malathion enantiomers with the minerals, at approximately the same amount.

To account for the loss of *R*-(*+*)- and *S*-(-)-malathion during sorption, the following equation was used, adapted from Oudou and Hansen (2002):

$$\mathrm{M_{min}} = \frac{\mathrm{M_{int}} - [\mathrm{Msol} + (\mathrm{M_{int}} \times \%\mathrm{loss})]}{\mathrm{M_{sorbent}}} \qquad 3.2$$

where M_{min} is the mass of malathion sorbed by the minerals (mg/kg), M_{int} is the initial malathion mass added (mg), M_{sol} is the malathion mass remaining in solution after sorption (mg), %loss is the loss determined for each concentration level for the water controls, and $M_{sorbent}$ is the mass of the sorbent (kg).

Enantiomerization of malathion

Chiral analysis of the sorption of *R*-(*+*)- and *S*-(-)-malathion to bentonite, calcite, and montmorillonite resulted in enantiomerization of both enantiomers to racemic mixtures after two hours as indicated by Figures 3.3-3.5. Enantiomerization also occurred after two hours contact time with diatomaceous earth and seashells (data not shown). To determine the impact of sorbent weight on enantiomerization, sorption

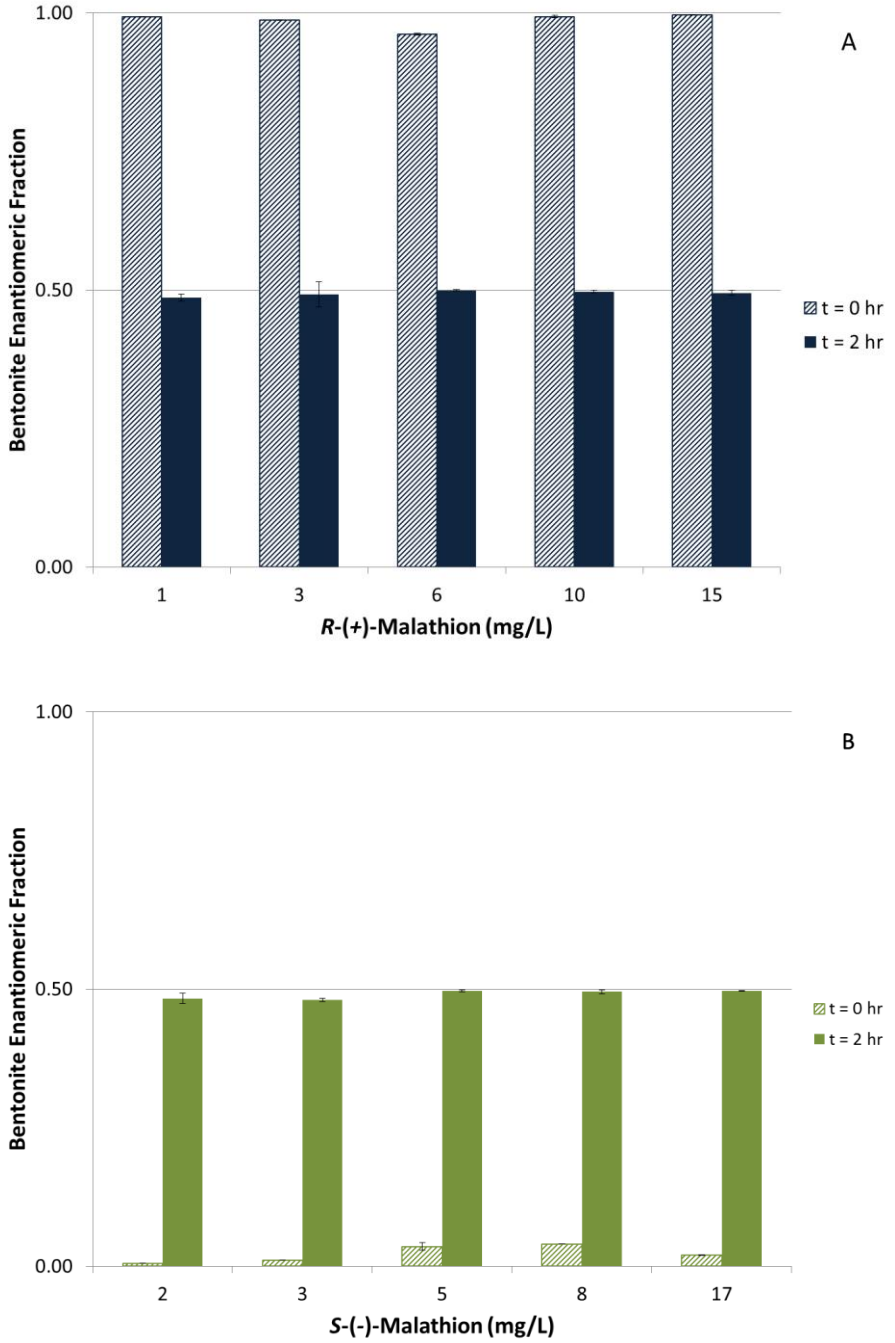


Figure 3.3: Enantiomerization of **A**) *R*-(*+*)-malathion and **B**) *S*-(-)-malathion after contact with bentonite for two hours. Error bars represent standard deviations of triplicates.

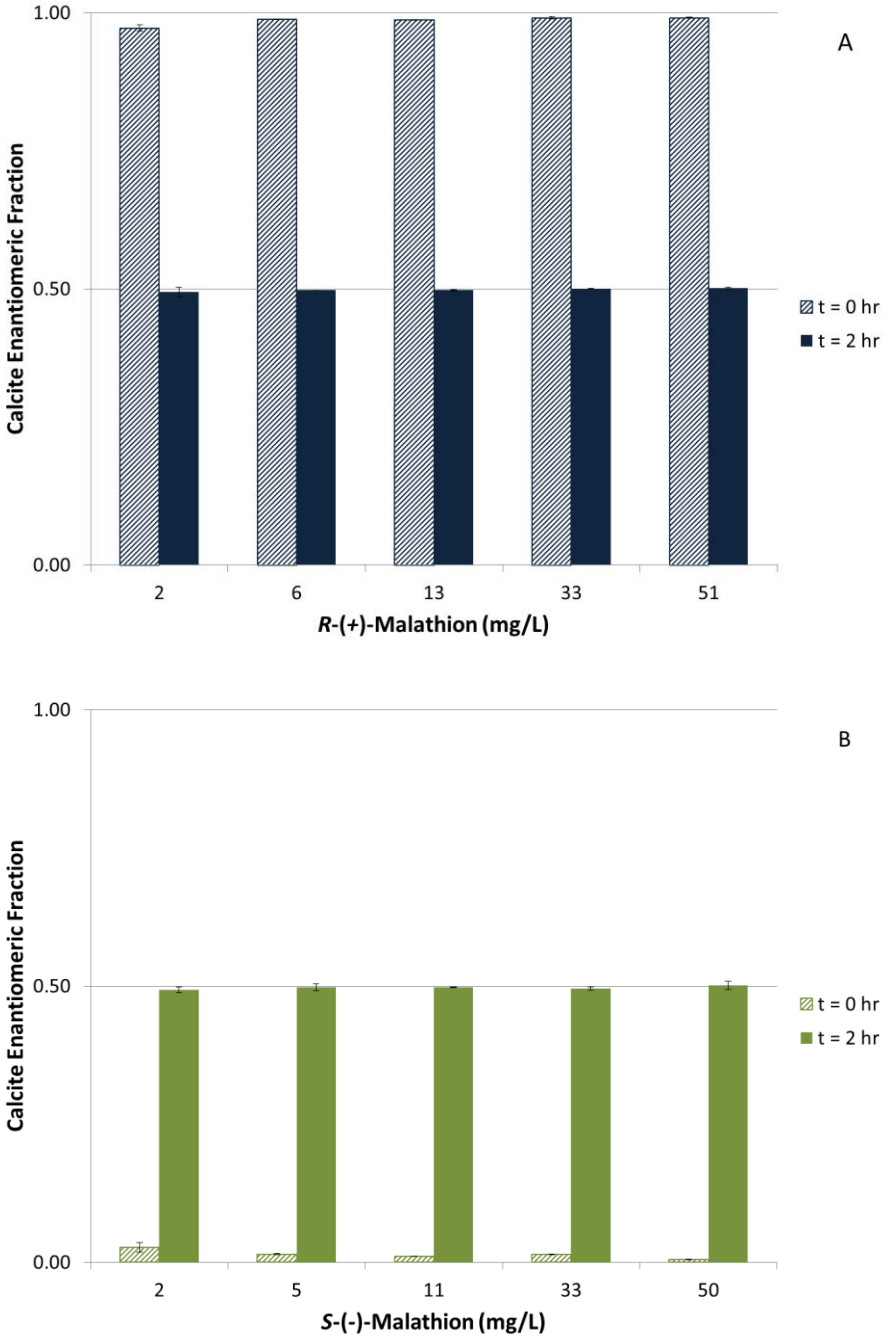


Figure 3.4: Enantiomerization of **A**) *R*-(*+*)-malathion and **B**) *S*-(-)-malathion after contact with calcite for two hours. Error bars represent standard deviations of triplicates.

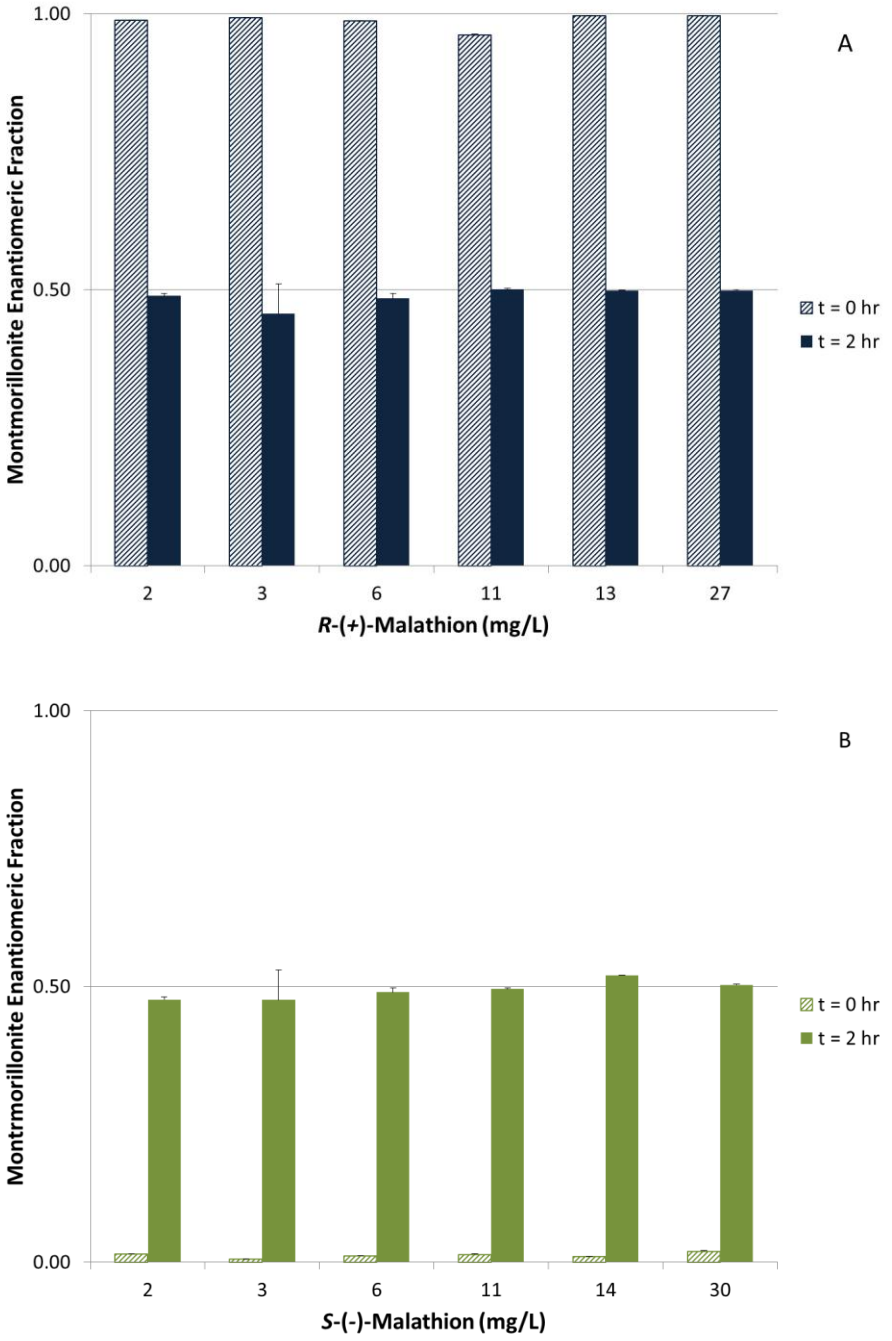


Figure 3.5: Enantiomerization of **A**) *R*-(*+*)-malathion and **B**) *S*-(-)-malathion after contact with montmorillonite for two hours. Error bars represent standard deviations of triplicates.

samples were prepared with 5, 12.5, and 25 mg of bentonite, vortexed for 5 sec and immediately centrifuged to separate the phases. Both malathion enantiomers racemized after contact with bentonite as shown in Figure B.5. To determine if the enantiomerization of *R*-(*+*)- and *S*-(-)-malathion occurred immediately after contact with the sorbent, samples were prepared using bentonite, calcite, diatomaceous earth, montmorillonite, and sea shells; vortexed briefly and immediately centrifuged to separate the phases. Analysis of the aqueous phase indicated complete enantiomerization to racemic of *R*-(*+*)-malathion for all sorbents, and partial enantiomerization for *S*-(-)-malathion as shown in

Figure B.6. Sorption controls using β-cyclodextrin indicated slight enantiomerization as mentioned previously, but not the complete enantiomerization observed with the mineral sorbents.

From the chiral results, enantiomerization of both enantiomers of malathion occurred for all sorbents and almost immediately after contact with the sorbent. There have been several reports of abiotic enantiomerization of pesticides in solvents such as enantiomerization of the pyrethroid pesticides cypermethrin and fenpropathrin in alcohols and water (Li et al., 2010; Nillos et al., 2009; Qin and Gan, 2007). However, this research presents the first known report of enantiomerization after contact with a surface. Li et al. (2010) hypothesized that enantiomerization of malathion, fenpropathrin, and phenthoate occurs due to the removal of H^+ on the chiral center and subsequent addition of H^+ forming the opposite enantiomer. The H^+ is acidic due to the

phosphorus and sulfur substituents which stabilize the carbanion and can be removed in water or undergo reactions with the mineral surface during sorption, as evidenced by this research. The lack of enantiomerization observed in the aprotic solvents hexane and acetone also provides evidence for the possibility of H^+ addition and removal as mechanism for enantiomerization. A hypothetical scheme of malathion enantiomerization adapted from Li et al (2010) is shown in Figure 3.6. The enantiomerization of malathion enantiomers indicates that application of enantiopure formulas of malathion would not be effective and not all chiral pesticides can be marketed as single enantiomers.

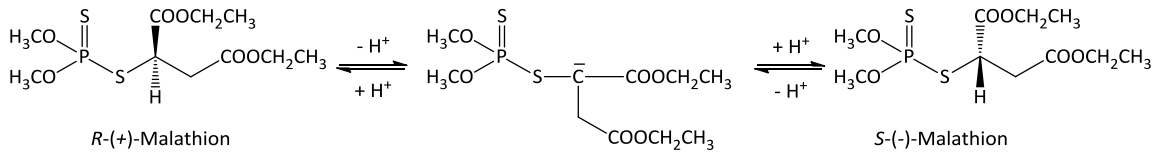


Figure 3.6: Hypothetical enantiomerization mechanism of malathion.

Enantiospecific sorption of malathion

Sorption equilibration time was chosen as two hours, which was not true equilibrium for the sorption of malathion enantiomers to the sorbents. As discussed in Chapter 2, malathion was not detected in the aqueous solution after eight hours of

contact with bentonite. To accurately quantify malathion enantiomer concentration in solution after sorption, the two hour time point was chosen to investigate sorption and determine if enantiomerization or enantioselective sorption occurred.

Sorption isotherms for sorption of *R*-(*+*)- and *S*-(-)-malathion to bentonite, calcite, and montmorillonite are shown in Figures B.7-B.9, along with malathion racemate isotherms discussed in Chapter 2. Although *R*-(*+*)- and *S*-(-)-malathion racemized after contact with the sorbent, sorption data were analyzed to determine if there were observable differences in sorption dependent on the starting enantiomer. "*Rac*-malathion" indicates the racemate was added as the sorbate and *R*-(*+*)- and *S*-(-)-malathion indicate either the *R*-(*+*)- or *S*-(-)-enantiomer was added as the sorbate, respectively. The racemic malathion sorption data were first presented in Chapter 2 and is presented here again along with the malathion enantiomers for comparison purposes. Bentonite sorption of *R*-(*+*)-malathion exhibits a L-type isotherm, similar to racemic malathion which indicates less available sorption sites as the sorbent surface is covered with sorbate. However, the sorption isotherm of *S*-(-)-malathion appears to be S-type, which results from cooperative absorption (Giles et al., 1974). Calcite and montmorillonite isotherms for the malathion enantiomers are S-type, in contrast to the L-type isotherms of the racemate. The differences in isotherm types possibly indicate different sorption mechanisms for the malathion enantiomers versus the racemate.

Distinct mechanisms for the sorption or enantiomers versus the racemate are possible due to the presence of a chiral environment provided by bentonite, calcite, and

montmorillonite. For chiral sorption to occur on a chiral surface, the three-point interaction model proposes that binding sites on the surface are selective to a specific enantiomer. Therefore, the chiral surface will selectively bind one enantiomer over another due to molecular interactions such as steric hindrance, hydrogen bonding, ionic and dipole interactions, and van der Waals interactions. These molecular interactions result in specific binding of an enantiomer to a chiral surface and no matter how the opposite enantiomer is rotated or positioned, three points of interactions are not achieved (Berthod, 2006). For example, chiral pesticides, such as mecoprop and dichlorprop, are known to undergo stereospecific interactions with enzymes, which are chiral and provide a surface for enantiospecific interactions to occur as reviewed by Muller and Kohler (2004). Since bentonite, calcite, and montmorillonite can display chirality, enantioselectivity resulting in distinct sorption mechanisms for the racemate and enantiomers is possible.

Malathion sorption data were fitted to the linear form of the Freundlich sorption model using the following equation, specifying two hours of equilibration:

$$\log C_S = \log K_{F,\,2hr} + n_{2hr} \log C_W \qquad 3.3$$

where C_s is the amount of malathion sorbed to the minerals (mg/kg), $K_{F,2hr}$ and n_{2hr} are the Freundlich constant and Freundlich exponent at two hours of contact, respectively, and C_w is the amount of malathion remaining in the aqueous phase after sorption

(mg/L). Freundlich isotherms are shown in Figures B.10-B.12 and the calculated parameters listed in Table 3.1 along with racemic malathion data from Chapter 2. The sorption data fit the Freundlich model with the exception of *R*-(*+*)-malathion sorption to calcite, which was only weakly correlated (r^2 = 0.4675). Freundlich n_{2hr} values were less than one for all sorbents, indicating that as malathion concentration increases, the free energies associated with sorption decreases (Schwarzenbach et al., 2003).

Table 3.1: Malathion Freundlich isotherm parameters.

Sorbent	Enantiomer	n_{2hr}	$K_{F,2hr}$	r^2
Bentonite	*R*-(*+*)-Malathion	0.775 ± 0.025	654 ± 1.05	0.9866
	S-(-)-Malathion	0.918 ± 0.049	613 ± 1.10	0.9760
	rac-Malathion	0.767 ± 0.034	724 ± 1.07	0.9752
Calcite	*R*-(*+*)-Malathion	0.526 ± 0.156	85.7 ± 1.55	0.4675
	S-(-)-Malathion	0.677 ± 0.094	78.2 ± 1.29	0.8139
	rac-Malathion	0.736 ± 0.056	87.3 ± 1.20	0.9311
Montmorillonite	*R*-(*+*)-Malathion	0.844 ± 0.038	254 ± 1.10	0.9694
	S-(-)-Malathion	0.941 ± 0.085	219 ± 1.20	0.8835
	rac-Malathion	0.907 ± 0.038	238 ± 1.10	0.9780

In comparing sorption capacity, malathion racemate and enantiomers were found to sorb the most to bentonite, followed by montmorillonite and calcite. Comparing malathion racemate and enantiomers to each sorbent resulted in no significant difference in n_{2hr} for montmorillonite calcite (Table B.1), but a significant difference was

observed for bentonite for *R*-(*+*)- and *S*-(-)-malathion (p = 0.0188) and *rac*- and *S*-(-)-malathion (p = 0.017). However, no significant difference was seen among $K_{F,2hr}$ for bentonite , calcite , and montmorillonite Table B.1) indicating all three sorbents had the same sorption capacity for racemic, *R*-(*+*)-, and *S*-(-)-malathion.

There have only been a few reports of enantiospecific sorption of pesticides in the literature, and none involving malathion (Matallo et al., 1998; Oravec et al., 2010; Zipper et al., 1998). Specific enantiomer sorption of mecoprop was found to be nonenantioselective to minerals including calcite and montmorillonite, with similar adsorption coefficients for *R*- and *S*-mecoprop (Zipper et al., 1998). Sorption of racemic mecoprop and dichlorprop and the *R*-enantiomers was also found to be nonenantioselective to soil, however only achiral analysis was used (Matallo et al., 1998). Sorption of mecoprop to humic acid and ash was determined to be slightly enantioselective (Oravec et al., 2010). In the literature, no indication of enantiomerization of mecoprop or dichlorprop was mentioned, but could possibly be a factor in sorption.

Conclusions

Individual malathion enantiomers were separated and collected by chiral HPLC and sorption to minerals was investigated by achiral and chiral analysis. Sorption of the malathion enantiomers was found to follow the Freundlich sorption model for calcite, bentonite, and montmorillonite, with the exception of *R*-(*+*)-malathion sorption to

calcite, which was only weakly correlated (r^2 = 0.4675). Freundlich exponent n_{2hr} values were statistically different for *R*-(*+*)- and *S*-(-)-malathion and for *rac*- and *S*-(-)-malathion for bentonite for the enantiomers, but no difference was seen with sorption of the enantiomers by calcite and montmorillonite . There was no statistical difference in the Freundlich constant ($K_{F,2hr}$) for all three sorbents for the enantiomers. Regardless of the malathion enantiomer added to the sorbents, complete enantiomerization occurred for bentonite, calcite, diatomaceous earth, montmorillonite, and seashells. Control samples using water or β-cyclodextrin indicated significant enantiomerization, but not complete conversion to equal amounts of enantiomers. Malathion enantiomers were also stable in the chiral HPLC eluent, which leads to the assumption that malathion enantiomerization is occurring due to surface interactions with the sorbents causing enantiomerization to occur faster with contact with the sorbents. It has been hypothesized that the proton present on the chiral center of malathion is relatively acidic due to carbanion stabilizing groups; the exchange of the proton with malathion and the sorbent could be causing enantiomerization in protic solvents (Li et al., 2010). To assess the impact of sorbent weight on enantiomerization of malathion, modified sorbent weight for bentonite also resulted in enantiomerization, indicating that enantiomerization is independent of the amount of sorbent present at the concentrations studied in this research. This is the first reported study of sorption of individual malathion enantiomers, and the first reported enantiomerization of enantiomers for a chiral pesticide when introduced to minerals. Further research is

needed to clarify the enantiomerization mechanism and understand the fate of malathion enantiomers after introduction to the environment, and to determine if this phenomenon is unique to malathion or is a potential mechanism for other chiral contaminants.

References

Asthagiri, A.; Hazen, R. M. An ab inito study of adsorption of alanine on the chiral calcite surface. *Molecular Simulation* **2007**, *33*, 343-351.

Berthod, A. Chiral recognition mechanisms. *Analytical Chemistry* **2006**, *78*, 2093-2099.

Bondarenko, S.; Gan, J. Degradation and sorption of selected organophosphate and carbamate insecticides in urban stream sediments. *Environmental Toxicology and Chemistry* **2004**, *23*, 1809-1814.

Bondy, S. C.; Harrington, M. E. L-Amino acids and D-glucose bind stereoselectively to a colloidal clay. *Science* **1979**, *203*, 1243-1244.

Bonner, W. A.; Kavasmaneck, P. R.; Martin, F. S. Flores, J. T. Asymmetric adsorption of alanine by quartz. *Science* **1974**, *186*, 143-144.

Buser, H. –R.; Muller, M. D.; Poiger, T.; Balmer, M. E. Environmental behavior of the chiral acetamide pesticide metalaxyl: Enantioselective degradation and chiral stability in soil. *Environmental Science and Technology* **2002**, *36*, 221-226.

Donia, A. M.; Atia, A. A.; Hussein, R. A.; Rashad, R. T. Efficient adsorption of malathion from different media using thermally treated kaolinite. *Desalination and Water Treatment* **2011**, *30*, 178-185.

Ellington, J. J.; Evans, J. J.; Prickett, K. B.; Champion Jr., W. L. High performance liquid chromatographic separation of enantiomers of organophosphorus pesticides on polysaccharide chiral stationary phases. *Journal of Chromatography A* **2001**, *928*, 145-154.

EPA. Reregistration eligibility decision for malathion **2006**, EPA 738-R-06-030.

Giles, C. H.; Smith, D.; Huitson, A. A general treatment and classification of the solute adsorption isotherm. *Journal of Colloid and Interface Science* **1974**, *47*, 755-765.

Gonzalez-Pradas, E.; Villafranca-Sanchez, M.; Fernandez Perez, M.; Socias Viciana, M. Adsorption of malathion from aqueous solution on homoionic bentonite samples. *Agrochimica* **1993**, *37*, 104-110.

Han, J. W.; Sholl, D. S. Enantiospecific adsorption of amino acids on hydroxylated quartz (0001). *Langmuir* **2009**, *25*, 10737-10745.

Han, J.W.; Sholl, D. S. Enantiospecific adsorption of amino acids on hydroxylated quartz ($10\bar{1}0$). *Physical Chemistry Chemical Physics* **2010**, *12*, 8024-8032.

Hazen, R. M.; Sholl, D. S. Chiral selection on inorganic crystalline surfaces. *Nature Materials* **2003**, *2*, 367-374.

Hazen, R. M.; Filley, T. R.; Goodfriend, G. A. Selective adsorption of L- and D-amino acids on calcite: Implications for biochemical homochirality. *PNAS* **2001**, *98*, 5487-5490.

Horvath, J. D.; Gellman, A. J. Naturally chiral surfaces. *Topics in Catalysis* **2003**, *25*, 9-15.

Julg, A.; Ozias, Y. Asymmetric adsorption of ethyliminium cation on kaolinite and L-homochirality of amino acids in proteins. *Journal of Molecular Structure (Theochem)* **1988**, *179*, 17-25.

Kulluru, P.P.; Das, B. S.; Panda, R. K. Evaluation of sorption and leaching potential of malathion and atrazine in agricultural soils of India. *International Journal of Environmental Research* **2010**, *4*, 75-90.

Li, Z.; Zhang, Z.; Zhang, L.; Leng, L. Isomer- and enantioselective degradation and chiral stability of fenpropathrin and fenvalerate in soils. *Chemosphere* **2009**, *76*, 509-516.

Li, Z.; Wu, T.; Li, Q.; Zhang, B.; Wang, W.; Li, J. Characterization of racemization of chiral pesticides in organic solvents and water. *Journal of Chromatography A* **2010**, *1217*, 5718-5723.

MacNamara, G.; Toth, S. J. Adsorption of linuron and malathion by soils and clay minerals. *Soil Science* **1970**, *109*, 234-239.

Matallo, M. Romero, E. Sanchez-Rasero, F. Adsorption of mecoprop and dichlorprop on calcerous and organic matter amended soils. *Journal of Environmental Science and Health, Part B: Pesticides Food Contaminants and Agricultural Wastes* **1998**, *33*, 51-66.

Messina, A.; Sinibaldi, M. CEC enantioseparations on chiral monolithic columns: A study of the stereoselective degradation of (*R*/*S*)-dichlorprop [2-(2,4-dichlorophenoxy) propionic acid] in soil. *Electrophoresis* **2007**, *28*, 2613-2618.

Mohan, K. R.; Ramesh, A.; Seshaiah, K. Studies on adsorption of selected organophosphorus pesticides by clays. *Indian Journal of Chemical Technology* **2001**, *8*, 278-280.

Muller, M. D.; Buser, H. –R. Conversion reactions of various phenoxyalkanoic herbicides in soil. 1. Enantiomerization and enantioselective degradation of the chiral 2-phenoxypropionic acid herbicides. *Environmental Science and Technology* **1997**, *31*, 1953-1959.

Muller, T. A.; Kohler, H.-P.E. Chirality of pollutants—effects on metabolism and fate. *Applied Microbiology and Biotechnology* **2004**, *64*, 300-316.

Nillos, M. G.; Qin, S.; Larive, C. Schlenk, D.; Gan, J. Epimerization of cypermethrin stereoisomers in alcohols. *Journal of Agricultural and Food Chemistry* **2009**, *57*, 6938-6943.

Oravec, M.; Simek, Z.; Holoubek, I. The effect of humic acid and ash on enantiomeric fraction change of chiral pollutants. *Colloids and Surfaces A: Physiochemical Engineering Aspects* **2010**, *359*, 60-65.

Oudou, H. C.; Hansen, H. C. B. Sorption of lambda-cyhalothrin, cypermethrin, deltamethrin and fenvalerate to quartz, corundum, kaolinite and montmorillonite. *Chemosphere* **2002**, *49*, 1285-1294.

Pal, O. R.; Vanjara, A. K. Removal of malathion and butachlor from aqueous solution by clays and organoclays. *Separation and Purification Technology* **2001**, *24*, 167-172.

Polec, I.; Cieslak, L.; Sledzinski, B.; Ksycinska, H. Simple syntheses of malathion and malaoxon enantiomers, and isomalathion diastereoisomers: Toxicity-configuration relationship. *Pesticide Science* **1998**, *53*, 165-171.

Qin, S.; Gan, J. Abiotic enantiomerization of permethrin and cypermethrin: Effects of organic solvents. *Journal of Agricultural and Food Chemistry* **2007**, *55*, 5734-5739.

Reist, M.; Testa, B.; Carrupt, P. A.; Jung, M.; Schurig, V. Racemization, enantiomerization, diastereomerization, and epimerization: Their meaning and pharmacological significance. *Chirality* **1995**, *7*, 396-400.

Romero, E.; Matallo, M. B.; Pena, A.; Sanchez-Rasero, F.; Schmitt-Kopplin, Ph.; Dios, G. Dissipation of racemic mecoprop and dichlorprop and their pure *R*-enantiomers in three calcareous soils with and without peat addition. *Environmental Pollution* **2001**, *111*, 209-215.

Sanchez Camazano, M.; Sanchez Martin, M. J. Factors influencing interactions of organophosphorus pesticides with montmorillonite. *Geoderma* **1983**, *29*, 107-118.

Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M. *Environmental Organic Chemistry*. **2003**, 2nd Edition. John Wiley and Sons, Inc.

Siffert, B.; Naidja, A. Stereoselectivity of montmorillonite in the adsorption and deamination of some amino acids. *Clay Minerals* **1992**, *27*, 109-118.

Sujatha, C. H.; Chacko, J. Malathion sorption by sediments from a tropical estuary. *Chemosphere* **1991**, *23*, 167-180.

Sun, M; Liu, D.; Zhou, G.; Li, J.; Qiu, X.; Zhou, Z.; Wang, P. Enantioselective degradation and chiral stability of malathion in environmental samples. *Journal of Agricultural and Food Chemistry* **2012**, *60*, 372-379.

Uchimiya, M.; Wartelle, L. H.; Boddu, V. M. Sorption of triazine and organophosphorus pesticides on soil and biochar. *Journal of Agricultural and Food Chemistry* **2012**, *60*, 2989-2997.

Wedyan, M.; Preston, M. R. Isomer-selective adsorption of amino acids by components of natural sediments. *Environmental Science and Technology* **2005**, *39*, 2115-2119.

Wolf, C. *Dynamic Stereochemistry of Chiral Compounds-Principles and Applications* **2008**, The Royal Society of Chemistry, Cambridge, United Kingdom.

Wolfe, N. L.; Zepp, R. G.; Baughman, G. L.; Gordon, J. A. Kinetic investigation of malathion degradation in water. *The Bulletin of Environmental Contamination and Toxicology* **1975**, *13*, 707-713.

Zhang, A.; Xie, X.; Ye, J.; Lin, C.; Hu, X. Stereoselective toxicity of malathion and its metabolites, malaoxon and isomalathion. *Environmental Chemistry Letters* **2011**, *9*, 369-373.

Zipper, C.; Suter, M, J.; Haderlein, S. B.; Gruhl, M.; Kohler, H. P. Changes in the enantiomeric ratio of (*R*)- to (*S*)-mecoprop indicate in situ biodegradation of this chiral herbicide in a polluted aquifer. *Environmental Science and Technology* **1998**, *32*, 2070-2076.

CHAPTER 4

SORPTION OF RACEMIC METALAXYL AND METALAXYL-M TO MINERALS

Abstract

Sorption of racemic metalaxyl and metalaxyl-M to minerals was investigated by chiral and achiral HPLC analysis. Metalaxyl-M consisted of 97% *R*-(-)-metalaxyl and 3% *S*-(*+*)-metalaxyl and sorption was accounted for each enantiomer by applying the enantiomeric fractions (EF) to sorption calculations of metalaxyl-M. Both forms of metalaxyl sorbed significantly to bentonite and montmorillonite, but no sorption to calcite and kaolinite was observed. Sorption results were fit to the Freundlich model, with $n > 1$ for bentonite and montmorillonite and K_F inferring metalaxyl sorption capacity was higher for bentonite. Sorption of *R*-(-)-metalaxyl from metalaxyl-M was higher than sorption of *rac*-metalaxyl, but *S*-(*+*)-metalaxyl sorption was the highest. Chiral analysis indicated nonenantioselective sorption of *rac*-metalaxyl, with racemic EFs. However, metalaxyl-M EFs increased after contact with bentonite and montmorillonite. Due to the significant sorption of metalaxyl-M to bentonite and montmorillonite, and the relatively small amount of *S*-(*+*)-metalaxyl present in the formulation, some error in EF determination may be possible, causing the changes in EF. However, since sorption capacity of bentonite and montmorillonite for *R*-(-)-metalaxyl was almost four times higher than *rac*-metalaxyl, apparently enantioselective sorption of *R*-(-)-metalaxyl in metalaxyl-M or enantiomerization occurred. EF also increased for

metalaxyl-M after contact with calcite, and EF decreased for one concentration level after contact with kaolinite. No sorption of calcite and kaolinite was observed for metalaxyl, therefore it is possible that some type of enantiomerization is taking place when in contact with the sorbent. This study presents the first investigation of enantioselective sorption of metalaxyl and metalaxyl-M.

Introduction

Metalaxyl is a chiral selective systemic fungicide and with an asymmetrical carbon at the carboxyl moiety, resulting in one pair of enantiomers. The fungicidal activity of metalaxyl is entirely due to *R*-(-)-metalaxyl; mefenoxam or metalaxyl-M is currently marketed as a chiral switch (replacement of a single enantiomer instead of racemic mixture), containing 96.5% *R*-(-)-metalaxyl and 2.5% *S*-(*+*)-metalaxyl (Buser et al., 2002). By marketing metalaxyl as primarily *R*-(-)-metalaxyl, the effective application rate is reduced by 50% versus racemic metalaxyl.

Sorption of metalaxyl to environmental surfaces has been studied extensively (Andrades et al., 2001; Andrades et al., 2004; Arias et al., 2006; de Wilde et al., 2008; Fernandes et al., 2003; Fernandes et al., 2006; Marin-Benito et al., 2009; Monkiedje and Spiteller, 2002; Rodriquez-Cruz et al., 2006; Saha and Sukul, 1997; Sanchez-Martin et al., 2006; Sharma and Awasthi, 1997; Sharom and Edgington, 1982; Sukop and Cogger, 1992; Sukul and Spiteller, 2000b; Sukul and Spiteller, 2001). Metalaxyl is moderately water soluble (8400 mg/L, Sukul and Spiteller, 2000a), but has been found to

significantly sorb to several types of soils as well as clays such as montmorillonite and kaolinite (Andrades et al., 2001; Andrades et al., 2004; de Wilde et al., 2008; Monkiedje and Spiteller, 2002; Sanchez-Martin et al., 2006; Sharma and Awasthi, 1997; Sharom and Edgington, 1982). However, there is little sorption research involving metalaxyl-M, the current form of metalaxyl on the market today with only one report of sorption of metalaxyl-M published. The report by Monkiedje and Spiteller (2002) used racemic metalaxyl, mefenoxam (48% mefenoxam), and a formulated metalaxyl (24% metalaxyl) to determine sorption to two soils and racemic metalaxyl sorbed more than the mefenoxam and the formulated metalaxyl on both soils. The difference in sorption was attributed to the additives in the mefenoxam and formulated metalaxyl; the inactive ingredients may compete for sorption sites with metalaxyl, and therefore, sorb less than purely racemic metalaxyl.

Formulations of enantiopure pesticides, such as metalaxyl-M, are emerging rapidly, and isomerization of these pesticides in the environment is critical to their efficacy. Isomerization, including racemization and enantiomerization, of enantiopure pesticides is not desirable; formation of the opposite enantiomer can reduce the toxicity to target organisms or increase toxicity to non-target organisms. Racemization is the irreversible transformation of one enantiomer into its antipode, whereas enantiomerization is the reversible interconversion of enantiomers (Reist et al., 1995; Wolf, 2008). Sorption and degradation processes could possibly induce isomerization and deserves more attention in the environmental literature. The first report of

isomerization of a pesticide during sorption has been reported in Chapter 3 of this dissertation, involving the racemization of malathion after contact with bentonite, calcite, diatomaceous earth, montmorillonite, and seashells. Enantioselective degradation research has also proven the isomerization of the chiral herbicides mecoprop and dichlorprop in soil (Messina and Sinibaldi, 2007; Muller and Buser, 1997; Romero et al., 2001). Isomerization can also occur in solvents as shown with the pyrethroids cypermethrin and fenpropathrin, and the organophosphorus pesticides malathion and phenthoate (Li et al., 2010; Nillos et al., 2009; Qin and Gan, 2007). Therefore, isomerization of chiral pesticides should be examined more thoroughly, particularly when a chiral switch such as metalaxyl is used in the environment. Enantiomerization and racemization are commonly used interchangeably, but in this dissertation, enantiomerization is used to define interconversion of enantiomers resulting in a mixture that may or may not be racemic.

The current consensus in environmental literature is that enantioselective processes are solely biotic, that is abiotic processes such as sorption are nonenantioselective. Nevertheless, the concept of chiral sorption, which is preferential sorption of a specific enantiomer, is essential to homochirality research. Homochirality, vital to origin-of-life theories, is the existence of only one enantiomer in the environment, such as L-amino acids and D-sugars. To understand the occurrence of homochirality, enantioselective amino acid sorption has been studied extensively, proving selective sorption of enantiomers to environmental surfaces (Asthagiri and

Hazen, 2007; Bondy and Harrington, 1979; Bonner et al., 1974; Han and Sholl, 2009; Han and Sholl, 2010; Hazen et al., 2001; Siffert and Naida, 1992; Wedyan and Preston, 2005). However, there has been little research investigating the possibility of enantioselective sorption of pesticides (Matallo et al., 1998; Oravec et al., 2010; Zipper et al., 1998).

Enantioselective sorption requires that the surface and the substrate be chiral, and at least three interaction points are required (Berthod, 2006; Hazen and Sholl, 2003). Environmental matrices such as quartz and kaolinite are chiral; calcite is achiral but can display chiral crystal faces and montmorillonite can display chirality in the stacking of its structural units (Hazen and Sholl, 2003; Horvath and Gellman, 2003; Julg and Ozias, 1988; Siffert and Naidja, 1992). Soils and sediment contain minerals and organic matter, possibly containing many stereogenic centers for chiral sorption possibilities. Enantioselective degradation of metalaxyl is assumed to be solely biotic, refuting the possibility of enantioselective sorption (Buerge et al., 2003; Buser et al., 2002 Marucchini and Zadra, 2002). My research aims to test the hypothesis of enantioselective sorption and isomerization of *rac*-metalaxyl and metalaxyl-M with minerals, using achiral and chiral HPLC analysis.

Materials and Methods

Chemicals and materials

Racemic metalaxyl (*rac*-metalaxyl, 99.5%) and mefenoxam (metalaxyl-M, 99.5%) were obtained from Chem Service (West Chester, PA). HPLC grade hexanes and

acetonitrile were obtained from Fisher Chemicals (Fair Lawn, NJ) and reagent alcohol consisting of 90:5:5 ethanol: isopropanol: methanol was obtained from Richard-Allan Scientific (Kalamazoo, MI). Deionized water (> 18 MΩ) was used for all experiments. Montmorillonite (SWy-2) and kaolinite (KGa-1b) were obtained from the Clay Minerals Society (West Lafayette, IN) and calcite crystals (0.2-0.5 cm) were obtained from Alfa Aesar (Ward Hill, MA). Bentonite was obtained from Spectrum Chemicals (New Brunswick, NJ) and all sorbents were used as received with no additional treatment. The chiral stationary phase cellulose tris-(4-methylbenzoate) was donated by Chiral Technologies (West Chester, PA) and β-cyclodextrin was obtained from Sigma Aldrich (St. Louis, MO).

Racemic metalaxyl and metalaxyl-M stability experiments

Aqueous solutions of *rac*-metalaxyl (25 mg/L) and metalaxyl-M (21 mg/L) were placed in 20 mL centrifuge tubes and placed on a wrist-action shaker protected from light. The experiment was conducted for 11 days in triplicate and aliquots were removed periodically to analyze by achiral and chiral analysis.

Sorption experiments

Separate batch sorption experiments were conducted using initial aqueous concentrations of *rac*-metalaxyl at 5, 10, 15, 20, and 25 mg/L and of metalaxyl-M at 4, 8, 12, 16, 20 mg/L. Metalaxyl concentration ranges were determined from a

comprehensive literature review indicating that for metalaxyl sorption experiments the initial aqueous concentrations typically ranged from 0.25-50 mg/L, therefore metalaxyl concentrations were selected to fall within this range (Andrades et al., 2004; Arias et al., 2006; Marin-Benito et al., 2009; Monkiedje and Spiteller, 2002; Rodriquez-Cruz et al., 2006; Sanchez-Martin et al., 2006; Sharma and Awasthi, 1997; Sukul and Spiteller, 2001). A 10 mL aliquot of each solution was added to a Pyrex test tube containing 50 mg of sorbent and all experiments were conducted in triplicate. Control samples either containing only water, chiral stationary phase cellulose tris-(4-methylbenzoate), β-cyclodextrin (1 g due to water solubility of β-cyclodextrin) were prepared as well. Equilibration time for metalaxyl sorption experiments was typically 24-48 hours, and a time study was conducted to verify equilibrium. Sorption samples were prepared in duplicate using bentonite and 15 mg/L of *rac*-metalaxyl. *Rac*-metalaxyl appeared to sorb quickly, but no significant additional sorption was seen after 24 hours of equilibration and the enantiomeric fractions (EFs) remained racemic (Table C.1). Therefore an equilibration time of 24 hours was used for all sorption samples. Samples and controls were vortexed briefly, mixed on a wrist-action shaker for 24 hours, and centrifuged at 1500 rpm for 15 min to separate the phases. The aqueous phase was analyzed by achiral analysis for quantitation and chiral analysis for EF determination. Sorption of metalaxyl was determined using the following equation:

$$\mathrm{M_{min}} = \frac{\mathrm{M_{int}} - \mathrm{Msol}}{\mathrm{M_{sorbent}}} \qquad 4.1$$

where M_{min} is the mass of metalaxyl sorbed by the minerals (mg/kg), M_{int} is the initial metalaxyl mass added (mg), M_{sol} is the metalaxyl mass remaining in solution after sorption (mg), and $M_{sorbent}$ is the mass of the sorbent (kg). *R*- and *S*-(*+*)-metalaxyl sorption from metalaxyl-M was determined by applying the EF to the sorbent calculations using the following equation for *R*-(-)-metalaxyl:

$$\mathrm{M_{min}} = \frac{[\mathrm{M_{int}}\,(1 - \mathrm{EFint})] - [\mathrm{Msol}\,(1 - \mathrm{EFsol})]}{\mathrm{M_{sorbent}}} \qquad 4.2$$

and the following equation for *S*-(*+*)-metalaxyl:

$$\mathrm{M_{min}} = \frac{(\mathrm{M_{int}} - \mathrm{EFint}) - (\mathrm{Msol} - \mathrm{EFsol})}{\mathrm{M_{sorbent}}} \qquad 4.3$$

where EF_{int} is the EF of the initial metalaxyl-M solution before adding to the sorbate and EF_{sol} is the EF of the final solution after sorption.

Achiral HPLC analysis

Achiral analysis was performed on a Waters Alliance 2690 Separation HPLC equipped with a 2996 PDA detector and Empower Pro chromatography software (Waters Corporation, Milford, MA). The column was a Zorbax SB-C18 rapid resolution column, 3.5 µm particle size, 4.6 x 100 mm (Agilent Technologies, Wilmington, DE). The

mobile phase consisted of 50:50 deionized water: acetonitrile at 1 mL/min and the run time was 6 min. The injection volume was 50 µL and metalaxyl absorbance was measured at 210 nm, with metalaxyl eluting at approximately 3 min (Figure C.1). Metalaxyl concentrations were quantified using a linear regression plot through the origin using five concentration levels ranging from 0.5-50 mg/L (r^2 = 0.9999) and LOD = 0.0033 mg/L. For achiral analysis, a portion of the aqueous layer was filtered through a 0.45 µm hydrophobic PFTE syringe filter into a HPLC autosampler vial and analyzed. Metalaxyl recoveries were > 98% verifying the extraction process was valid. Achiral analysis was used for quantitative analysis of sorption, considered as a loss of malathion from solution, with no consideration of chirality.

Chiral HPLC analysis

Chiral analysis was adapted from Ellington et al. (2001) using a 4.6 x 250mm Chiralcel®OJ® column packed with cellulose tris-(4-methylbenzoate) coated on a 10 µm silica gel substrate (Chiral Technologies, West Chester, PA) a Perkin Elmer Series 200 HPLC equipped with UV detection and TotalChrom 6.3.2 chromatography software (Perkin Elmer, Waltham, MA). The mobile phase consisted of 90:10 hexane: reagent alcohol at 0.9 mL/min, with 50 µL injection volume, 20^{o}C column temperature, and the Peltier autosampler at 10^{o}C. Metalaxyl absorbance was measured at 210 nm and the elution order was confirmed from metalaxyl-M as *S*-(*+*)-metalaxyl eluting first at approximately 8 min followed by *R*-(-)-metalaxyl at 10 min (Figures C.2-C.3). Metalaxyl-

M was determined to be approximately 97% *R*-(-)-metalaxyl and 3% *S*-(*+*)-metalaxyl. The LOD was 0.003 mg/L for *R*-(-)-metalaxyl and 0.0105 mg/L for *S*-(*+*)-metalaxyl. Enantiomeric fractions were calculated using the following equation:

$$\mathrm{EF} = \frac{\text{S-(+)}}{\text{R-(-)} + \text{S-(+)}} \qquad 4.4$$

where *R*-(-) and *S*-(*+*) represent the peak areas of the metalaxyl enantiomers, respectively. For chiral analysis, an aliquot of the aqueous layer was extracted with 2 mL of hexane and transferred to a HPLC vial for analysis. Recoveries of *R*-(-)- and *S*-(*+*)-metalaxyl for both *rac*- and metalaxyl-M indicated no change in EF, and verified the enantiomer stability during chiral analysis. EFs for the *rac*-metalaxyl standard varied from 0.47-0.52 and metalaxyl-M EF = 0.028-0.031. Chiral analysis was used to calculate EFs to determine if enantiomerization or enantioselective sorption occurred for malathion.

Statistical analysis

Statistical analysis for rate constants and Freundlich isotherm parameters was performed with R statistical software (R Foundation for Statistical Computing, 2012) using regression analysis with a qualitative predictor for the enantiomeric form of metalaxyl by comparing the slopes (rate constants or Freundlich exponent *n*) or intercepts (Freundlich capacity factor K_F) with $\alpha = 0.05$. Statistical analysis for EFs was

performed using single factor ANOVA in Microsoft Excel (Microsoft Office 2010), using $\alpha = 0.05$. P values are reported throughout the results and discussion and $p < 0.05$ is determined to be statistically significant whereas $p > 0.05$ is not statistically significant. EF results are presented with standard deviations whereas the rate constant, half-life, and Freundlich isotherm parameters are presented with standard error for linear regression analysis.

Results and Discussion

Stability experiments

No degradation of *rac*-metalaxyl and metalaxyl-M was seen with incubation in water after 11 days (Figure C.4). However, chiral analysis indicated a change in EF as shown in Figure 4.1. Enantiomeric fractions for both *rac*-metalaxyl and metalaxyl-M increase, indicating the formation of *S*-(*+*)-metalaxyl. The metalaxyl-M EF is moving toward racemic, whereas the EF for *rac*-metalaxyl is moving away from racemic. The change in EF is interesting because no degradation of metalaxyl was observed by achiral analysis, which suggests abiotic isomerization is taking place in water.

Sorption of racemic metalaxyl and metalaxyl-M

Controls containing only water confirmed the stability of *rac*-metalaxyl and metalaxyl-M for 24 hours. Only one concentration level, 15 mg/L for *rac*-metalaxyl

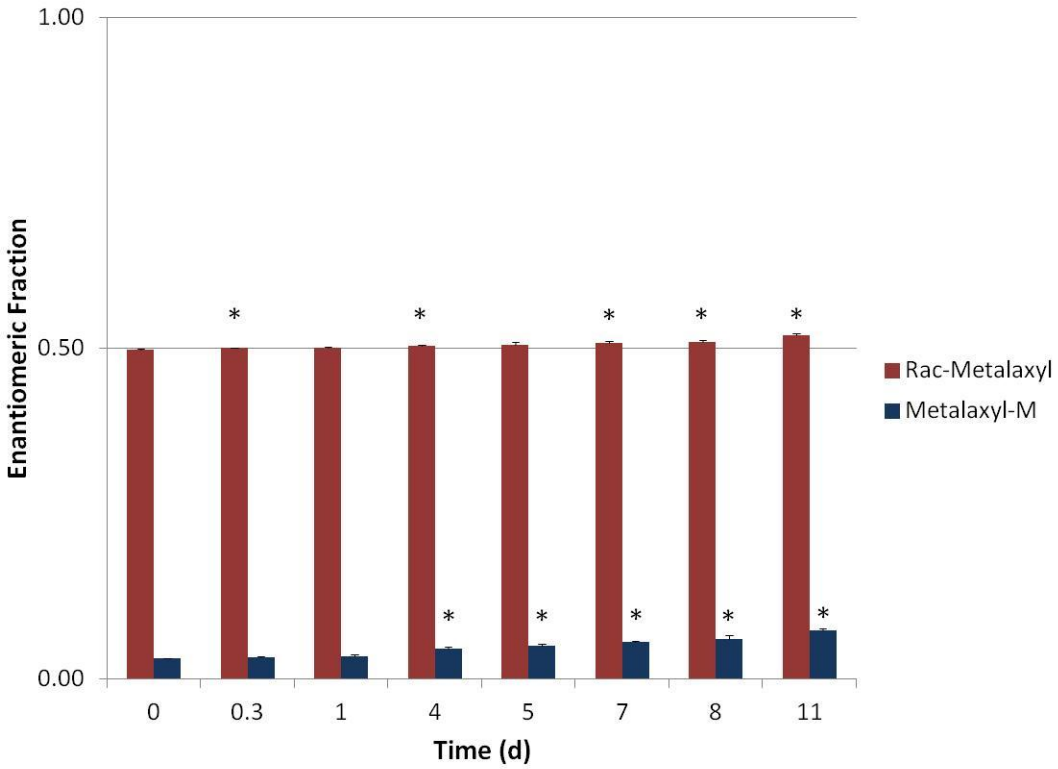


Figure 4.1: Enantiomeric fraction change of *rac*-metalaxyl and metalaxyl-M in water. Error bars represent standard deviations of triplicates. A "*" indicates that the difference is statistically significant ($p < 0.05$) from $t = 0$ d.

decreased in concentration after 24 hours, resulting in only a 4% loss of metalaxyl (Figure C.5). No concentration loss was observed for metalaxyl-M (Figure C.6). Enantiomeric fractions remained stable at EF = 0.50 ± 0.00 for metalaxyl and EF = 0.03 ± 0.00 (Figures C.7-C8). Sorption controls using the chiral stationary phase cellulose tris-(4-methylbenzoate) indicated approximately 15% loss of *rac*-metalaxyl and metalaxyl-M, but no significant change in EF ($p = 0.93$; $p = 0.88$).

Rac-metalaxyl and metalaxyl-M were found to sorb to bentonite and montmorillonite, but not to calcite and kaolinite. Aqueous phase recoveries of *rac*-metalaxyl and metalaxyl-M were > 95% for all concentration levels for calcite and kaolinite, indicating that no sorption occurred. Sorption isotherms were plotted for *rac*-metalaxyl and the *R*-(-)- and *S*-(*+*)-metalaxyl sorption of metalaxyl-M in Figures 4.2-4.3. *S*-(*+*)-metalaxyl sorption is very small compared to *rac*- and *R*-(-)-metalaxyl due to the small amount (3%) present in metalaxyl-M. Sorption of *rac*-metalaxyl and *S*-(*+*)-metalaxyl to bentonite and montmorillonite demonstrated S-type curves, which results from cooperative adsorption. On the contrary, *R*-(-)-metalaxyl sorption demonstrated L-type curves indicating that as concentration increases, sorption sites are less available as the sorbent surface is covered with the sorbate (Giles et al., 1974). The differences in the isotherm types for *rac*-metalaxyl and *R*-(-)-metalaxyl from metalaxyl-M seem to indicate different sorption mechanisms.

Distinct mechanisms for the sorption or enantiomers versus the racemate are possible due to the presence of a chiral environment provided by bentonite and

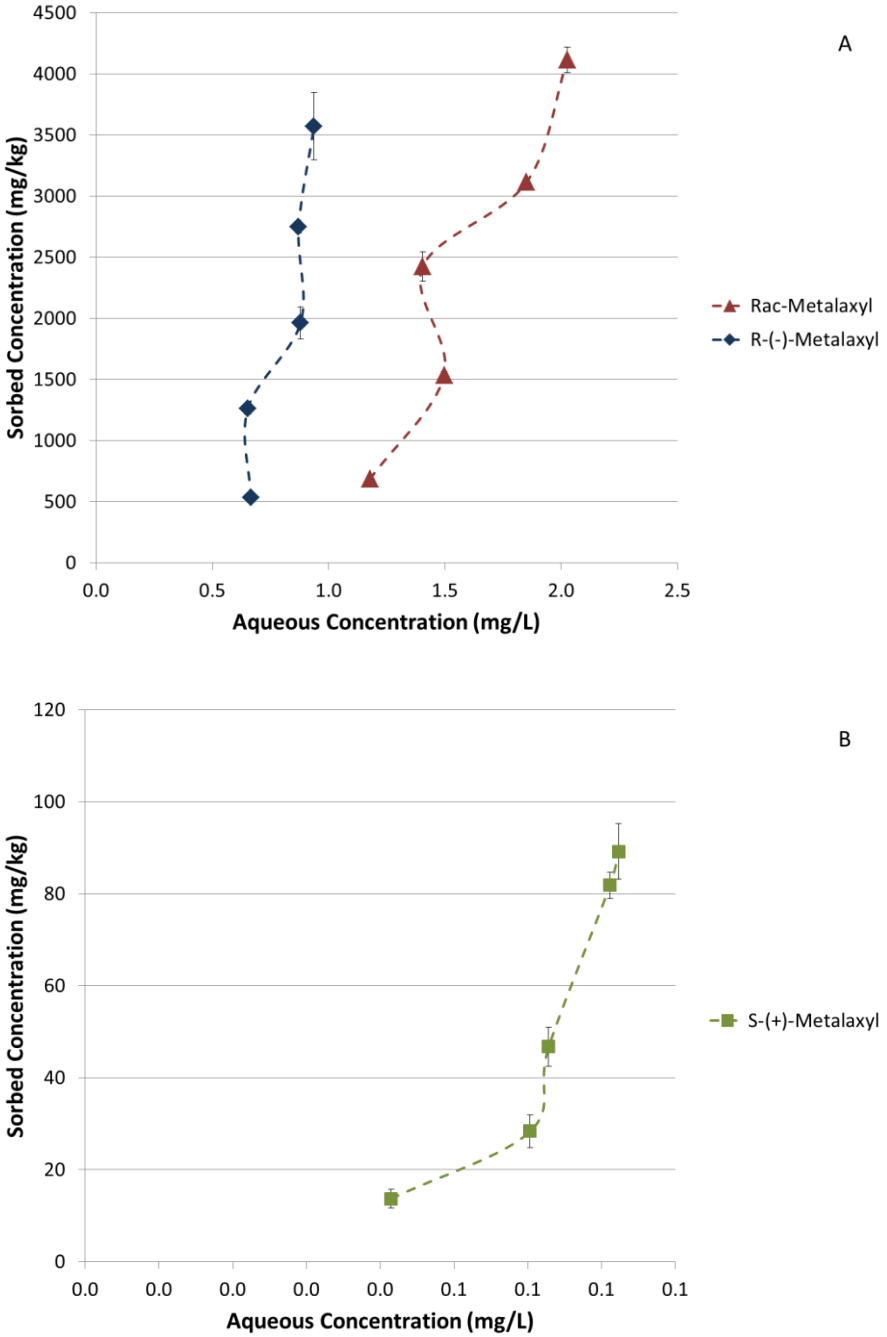


Figure 4.2: Bentonite sorption isotherms of **A**) *rac*-metalaxyl and *R*-(-)-metalaxyl and **B**) *S*-(*+*)-metalaxyl. Dotted lines represent isotherm shapes and do not represent model fits. Error bars represent standard deviations of triplicates.

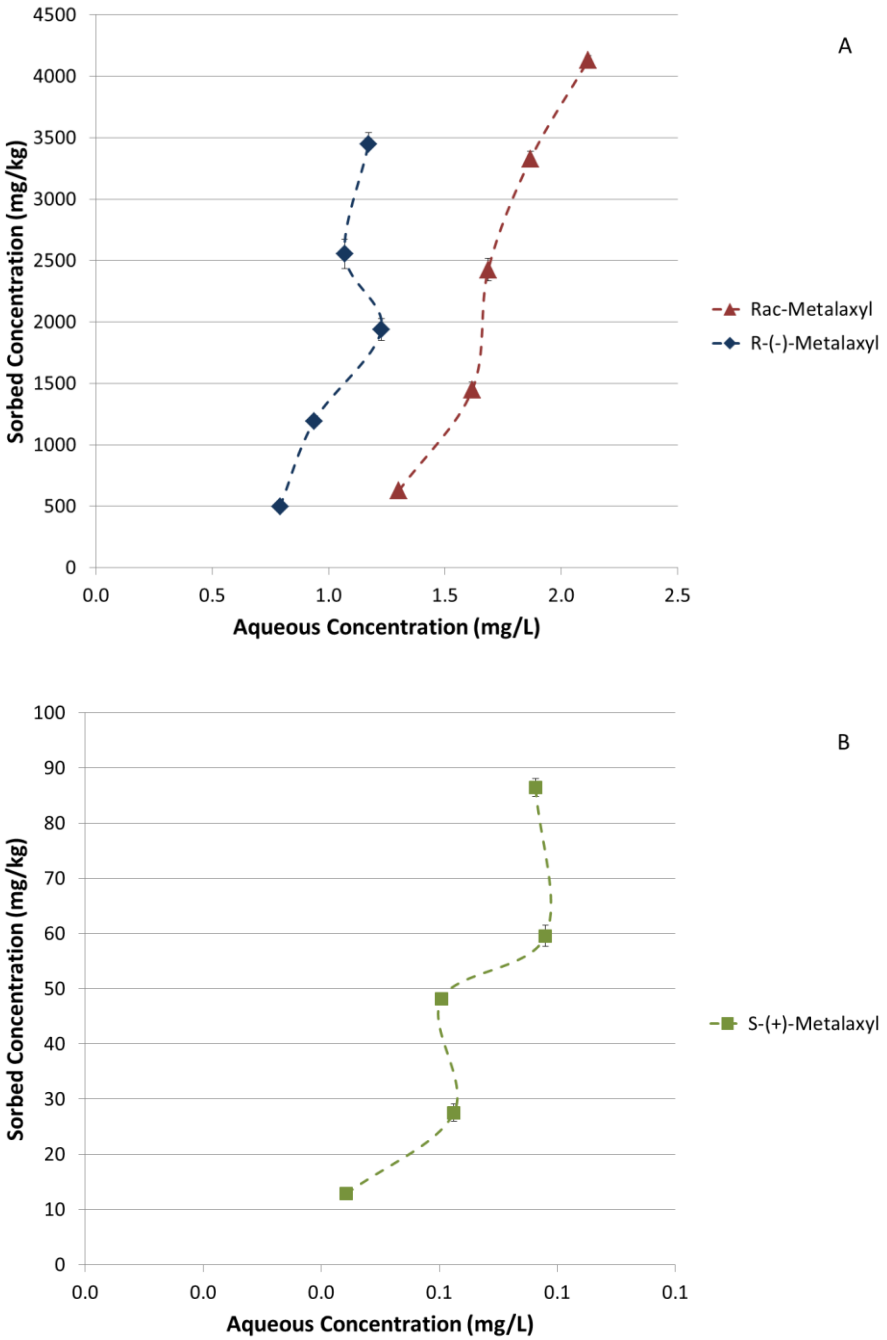


Figure 4.3: Montmorillonite sorption isotherms of **A**) *rac*-metalaxyl and *R*-(-)-metalaxyl and **B**) *S*-(*+*)-metalaxyl. Dotted lines represent isotherm shapes and do not represent model fits. Error bars represent standard deviations of triplicates.

montmorillonite. For chiral sorption to occur on a chiral surface, the three-point interaction model proposes that binding sites on the surface are selective to a specific enantiomer. Therefore, the chiral surface will selectively bind one enantiomer over another due to molecular interactions such as steric hindrance, hydrogen bonding, ionic and dipole interactions, and van der Waals interactions. These molecular interactions result in specific binding of an enantiomer to a chiral surface and no matter how the opposite enantiomer is rotated or positioned, three points of interactions are not achieved (Berthod, 2006). For example, chiral pesticides, such as mecoprop and dichlorprop, are known to undergo stereospecific interactions with enzymes, which are chiral and provide a surface for enantiospecific interactions to occur as reviewed by Muller and Kohler (2004). Since bentonite and montmorillonite can display chirality, enantioselectivity resulting in distinct sorption mechanisms for the racemate and enantiomers are possible.

Sorption of metalaxyl to bentonite and montmorillonite as seen in this research is comparable to Andrades et al. (2004) and Sanchez-Martin et al. (2006) where the sorption of *rac*-metalaxyl to clay minerals, including montmorillonite, was found to be the L- or S-type isotherms. However, both reports indicated sorption of metalaxyl to kaolinite, which was not observed in this study. It is important to note that the kaolinite used in my research was a source clay from the Clay Minerals Society (West Lafayette, IN) which has been homogenized and thoroughly characterized. On the contrary, the kaolinite materials used in the reports mentioned previously originate from Spain and

were characterized by the authors (Andrades et al., 2004; Sanchez-Martin et al., 2006). The differences in materials may be attributable to the differences observed with metalaxyl sorption to kaolinite.

The metalaxyl sorption data was fit to the Freundlich model using the following equation:

$$\log C_S = \log K_F + n \log C_W \qquad 4.5$$

where C_s is the amount of metalaxyl sorbed to the minerals (mg/kg), K_F is the Freundlich constant, *n* is the Freundlich exponent, and C_w is the amount of metalaxyl remaining in the aqueous phase after sorption (mg/L). Freundlich isotherms for bentonite and montmorillonite are seen in Figures C.9-C.10 and the calculated Freundlich parameters are listed in Table 4.1, indicating a positive, but moderate fit to the model with r^2 = 0.5175-0.7888. The Freundlich exponent *n*, which is a measure of concentration dependence on sorption, were all greater than one and similar for the sorption of the racemate and enantiomers for bentonite and montmorillonite ($p > 0.08$, Table C.2), indicating that as metalaxyl concentration increases, the free energies associated with sorption increases as well. Andrades et al. (2004) and Sanchez-Martin et al. (2006) also reported $n > 1$ for montmorillonite sorption of *rac*-metalaxyl. The Freundlich constant K_F, which is a measure of sorption capacity, indicated bentonite had the highest sorption for *rac*-metalaxyl and metalaxyl-M. For both bentonite and montmorillonite sorption

Table 4.1: Metalaxyl Freundlich isotherm parameters.

Sorbent	Enantiomer	*n*	K_F	r^2
Bentonite	*rac*-Metalaxyl	2.41 ± 0.46	692 ± 1.26	0.6765
	R-Metalaxyl	3.56 ± 0.63	3802 ± 1.202	0.7095
	S-Metalaxyl	2.02 ± 0.54	12589 ± 4.6773	0.5175
Montmorillonite	*rac*-Metalaxyl	3.38 ± 0.48	339 ± 1.32	0.7888
	R-Metalaxyl	3.24 ± 0.60	1479 ± 1.122	0.6898
	S-Metalaxyl	2.28 ± 0.47	21380 ± 3.7154	0.6402

decreased in the order of *S*-(*+*)-metalaxyl, *R*-(-)-metalaxyl, and *rac*-metalaxyl. Calculated K_F were statistically different for the racemate and *R*-(-)-metalaxyl (p = 0.000006) and *rac*- and *S*-(*+*)-metalaxyl (p = 0.00002), but not *R*-(-)- and *S*-(*+*)-metalaxyl sorption to bentonite. Calculated K_F were also statistically different for all racemate and enantiomer comparisons for metalaxyl sorption to montmorillonite: (*R*-(-)- and *S*-(*+*)-metalaxyl, p = 0.0318; *rac*- and *R*-(-)-metalaxyl, p = 0.00007; *rac*- and *S*-(*+*)-metalaxyl, r = 0.00006). *S*-(*+*)-metalaxyl sorption to bentonite and montmorillonite appears to be much larger in comparison to *rac*- and *R*-(-)-metalaxyl, which could be due to analytical error detecting the *S*-(*+*)-metalaxyl due to the relatively small amount of *S*-(*+*)-metalaxyl present in metalaxyl-M. The K_F values are determined from the intercept of a log-log plot (Equation 4.5) and small changes in log K_F results in large changes in K_F. The *rac*-metalaxyl K_F values reported in this research are much higher than the K_F = 0.05-14.1 reported from Andrades et al. (2004) and Sanchez-Martin et al. (2006) indicating that clay minerals used in their research have a much smaller sorption capacity for metalaxyl.

It is interesting to note that the sorption of *rac*-metalaxyl was more than four times less than the sorption of metalaxyl-M to bentonite and montmorillonite. Only one report of sorption of *rac*-metalaxyl and metalaxyl-M appears in the literature, and indicates sorption of *rac*-metalaxyl is higher than metalaxyl-M to two soils, contrary to the results seen in this research (Monkiedje and Spiteller, 2002). The metalaxyl-M used by Monkiedje and Spiteller (2002) was a 48% technical formulation, and the difference in sorption of *rac*-metalaxyl and metalaxyl-M could be attributed to the additives in the metalaxyl-M formulation; the inactive ingredients may compete for sorption sites with metalaxyl, and therefore, sorb less than a purely racemic metalaxyl. This research used pure metalaxyl-M, which resulted in higher sorption than *rac*-metalaxyl.

Chiral analysis of sorption samples of *rac*-metalaxyl to bentonite, calcite, kaolinite, and montmorillonite indicated nonenantioselective sorption, with racemic EFs (Figure C.11). However, an increase in EF was observed for metalaxyl-M with bentonite and montmorillonite at all concentration levels as seen in Figure 4.4. The apparent change in EF could be attributed to the small amount of *S*-(*+*)-metalaxyl in metalaxyl-M; sorption for both enantiomers was significant to bentonite and montmorillonite, which could cause error in the quantitation of *S*-(*+*)-metalaxyl by chiral HPLC. However, *S*-(*+*)-metalaxyl was present in amounts above the LOD, and metalaxyl-M quality control standards indicated consistent EFs throughout analysis. EF increased as well for calcite, although sorption of metalaxyl-M was not observed. For kaolinite, the 10 mg/L concentration level indicated a decrease of EF from EF = 0.0278 (± 0.0007) to EF =

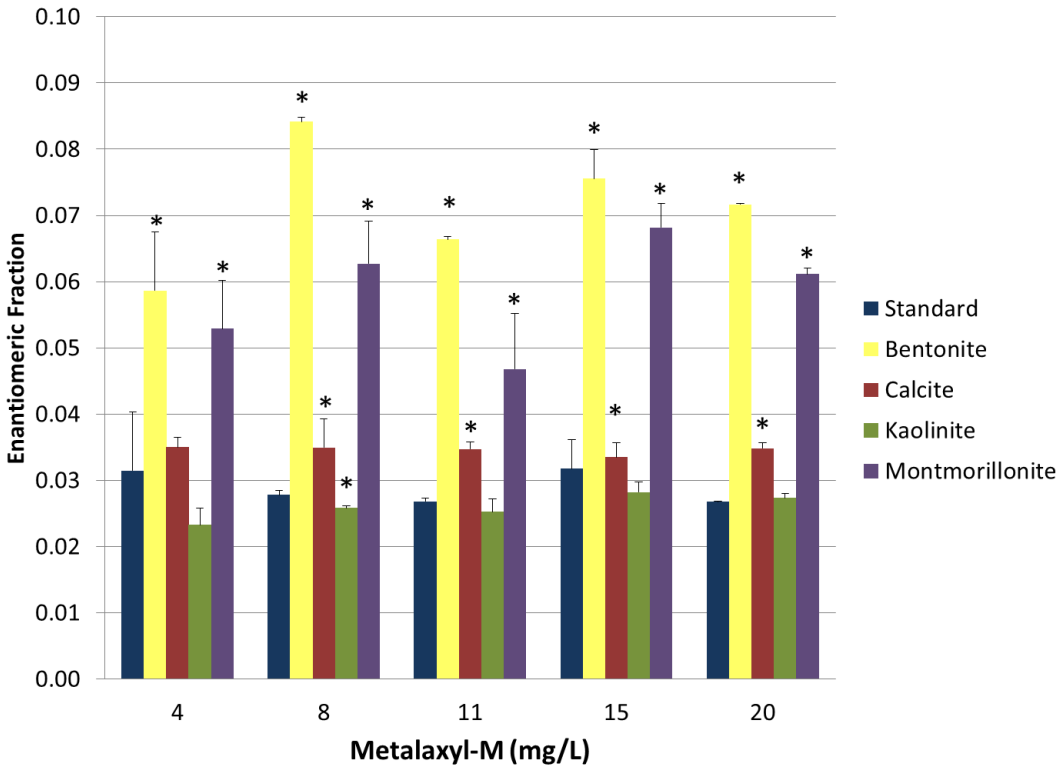


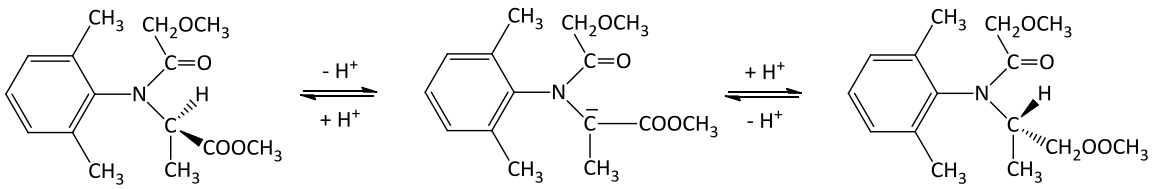
Figure 4.4: Increases in enantiomeric fractions for metalaxyl-M after sorption. Error bars represent standard deviations of triplicates. A "*" indicates that the difference is statistically significant ($p < 0.05$) from metalaxyl-M standard prior to addition to the sorbent.

0.0258 (± 0.0003), which was significant (p = 0.0087). EFs of sorption controls using only water or the chiral stationary phase remained constant, indicating the metalaxyl-M is either undergoing enantiomerization when in contact with all the sorbents or enantioselective sorption of *R*-(-)-metalaxyl is occurring with bentonite and montmorillonite. Since the metalaxyl-M sorption capacity of bentonite and montmorillonite is higher than *rac*-metalaxyl, it can be hypothesized that bentonite and montmorillonite have a higher affinity for *R*-(-)-metalaxyl. Therefore, enantioselective sorption of *R*-(-)-metalaxyl may be occurring when metalaxyl-M is in contact with bentonite and montmorillonite.

Metalaxyl sorption to soil and sediments has been thoroughly investigated, but this is the first report of enantioselective analysis of metalaxyl sorption and the first to investigate sorption of metalaxyl-M to minerals (Andrades et al., 2001; Andrades et al., 2004; Sharom and Edgington, 1982; Sukul and Spiteller, 2000b). Degradation of metalaxyl has been shown to be enantioselective, with varied enantiomer preferences in soils and sediments (Buerge et al., 2003; Buser et al., 2002; Chen and Liu, 2009; Jarman et al., 2005; Marucchini and Zadra, 2002; Monkiedje et al., 2003; Monkiedje et al., 2007; Muller and Buser, 1995; Wang et al., 2005). The enantiomerization of metalaxyl-M after sorption is novel in that there have been no reports of isomerization of metalaxyl enantiomers. Only two reports have used chiral analysis of metalaxyl enantiomers, and they are enantioselective degradation studies, which indicated no isomerization in soil (Buser et al., 2002; Marucchini and Zadra, 2002).

In the literature, there are only a few reports of enantiospecific sorption investigations. Mecoprop sorption was found to be sorbed in a nonenantioselective manner to minerals, including calcite and montmorillonite (Zipper et al., 1998). Racemic mecoprop and dichlorprop, along with their respective *R*-enantiomers were found to sorb nonenantioselectively to soil. However, only achiral analysis was used and the Freundlich constants were similar, thereby indicating nonenantioselective sorption. Sorption of mecoprop to humic acid and ash would found to be slightly enantioselective, but error analysis was not provided and the authors were vague on the significance of the change of EF after sorption (Oravec et al., 2010). In these reports, isomerization of mecoprop and dichlorprop was not mentioned, but could possibly be a factor in sorption. In Chapter 3 of this dissertation, malathion enantiomers were observed to racemize in water and during sorption with bentonite, calcite, diatomaceous earth, montmorillonite, and seashells. Abiotic enantiomerization has been observed for the pyrethroid insecticides cypermethrin and fenpropathrin (Li et al., 2010; Nillos et al., 2009; Qin and Gan, 2007) and the organophosphate pesticides malathion and phenthoate (Li et al., 2010; Sun et al., 2012). Li et al. (2010) hypothesized that the enantiomerization of malathion occurs due to proton extraction and removal at the chiral carbon, facilitated by carbanion stabilizing groups. In Chapter 3, an enantiomerization scheme for malathion was presented and a similar scheme for metalaxyl can be constructed as seen in Figure 4.5. The proton on the chiral carbon can

be acidic and relatively good leaving group due to the stabilization of the carbanion, and therefore, interaction with the sorbents can facilitate the proton exchange reactions.



R-(-)-Metalaxyl S-(+)-Metalaxyl

Figure 4.5: Hypothetical enantiomerization mechanism of metalaxyl.

The common consensus with the enantioselective degradation of metalaxyl is that the process is wholly biotic, that is abiotic processes such as sorption cannot be enantioselective (Buerge et al., 2003; Buser et al., 2002; Marucchini and Zadra, 2002). However, sorption of metalaxyl-M has indicated small but significant change in EF after contact with bentonite, calcite, kaolinite, and montmorillonite. No enantioselective biodegradation results have been published for metalaxyl-M however enantioselective biodegradation of *rac*-metalaxyl in soil has shown large changes of EF, such as EF = 0.49 to 0.94 after 60 d (Monkiedje et al., 2003) and EF = 0.50 to 0.02 after 55 d (Jarman et al., 2005). However, smaller changes in EF have also been observed for *rac*-metalaxyl enantioselective biodegradation in soil such as EF = 0.50 to 0.52 after 40 d and EF = 0.50

to EF 0.44 (approximate EFs and incubation times determined from Figure 4 in Buerge et al., 2003). These small changes of EF in Buerge et al. (2003) of only 0.02-0.06 are comparable to changes in metalaxyl-M EF after contact with bentonite (EF change 0.0423 ± 0.0140) and montmorillonite (0.0305 ± 0.0099). EF changes in calcite (EF change 0.0068 ± 0.0038) and kaolinite (0.0018 ± 0.0051) are smaller, but still significant indicating the enantiomerization of metalaxyl-M. The changes of EF for metalaxyl-M during contact with minerals questions the possibility of only biotic processes being enantioselective in environmental matrices.

Conclusions

Sorption of *rac*-metalaxyl and metalaxyl-M to bentonite, calcite, kaolinite, and montmorillonite was investigated by achiral and chiral analysis. Sorption of the individual enantiomers *R*-(-)- and *S*-(*+*)-metalaxyl of metalaxyl-M was determined by accounting for the enantiomeric fraction in sorption calculations. Both the racemate and the individual enantiomers were found to sorb significantly to bentonite and montmorillonite, but negligible sorption to calcite and kaolinite was observed. Sorption of the racemate and enantiomers was fit to the Freundlich sorption model for bentonite and montmorillonite. Freundlich *n* exponents were all above one and similar for sorption of the racemate or enantiomers to either bentonite or montmorillonite. The highest sorption capacity was found for *S*-(*+*)-metalaxyl, and higher sorption capacity of *R*-(-)-metalaxyl was also observed for bentonite and montmorillonite in comparison to

the sorption of the racemate. Sorption of the racemate to all sorbents was found to be nonenantioselective, as indicated by racemic enantiomeric fractions. However, sorption of metalaxyl-M indicated a change in EF after contact with the sorbents for bentonite, calcite, kaolinite, and montmorillonite. Changes in enantiomeric fraction due to interactions with bentonite and montmorillonite could be attributed to the small amount of *S*-(*+*)-metalaxyl causing an error in quantitation. Metalaxyl-M sorption capacity of bentonite and montmorillonite is higher than *rac*-metalaxyl, indicating the possibility of enantioselective sorption of *R*-(-)-metalaxyl in metalaxyl-M. Sorption of metalaxyl-M was not observed with calcite or kaolinite, but the enantiomer fraction slightly increased or decreased, respectively, indicating a possible reaction with the sorbent causing enantiomerization. Metalaxyl-M enantiomeric fractions were stable with either water or the HPLC chiral stationary phase cellulose tris-(4-methylbenzoate). This is the first known report of sorption of metalaxyl and metalaxyl-M using chiral analysis to assess the role of the individual enantiomers in sorption mechanisms. *R*-(-)-metalaxyl has been shown to sorb to a greater extent than the racemate, implying the possibility of enantioselective sorption. More research is needed to clarify the environmental fate and impact of metalaxyl enantiomers, since it is marketed as metalaxyl-M, consisting of primarily *R*-(-)-metalaxyl. As a novel concept, enantioselective sorption also should be investigated more thoroughly in assessing the fate of chiral pesticides in the environment.

References

Andrades, M. S.; Sanchez-Martin, M. J.; Sanchez-Camazano, M. Significance of soil properties in the adsorption and mobility of the fungicide metalaxyl in vineyard soils. *Journal of Agricultural and Food Chemistry* **2001**, *49*, 2363-2369.

Andrades, M. S.; Rodriguez-Cruz, M. S.; Sanchez-Martin, M. J.; Sanchez-Camazano, M. Effect of the modification of natural clay minerals with hexadecylpyridinium cation on the adsorption—desorption of fungicides. *International Journal of Environmental Analytical Chemistry* **2004**, *84*, 133-141.

Arias, M.; Paradelo, M.; Lopez, E.; Simal-Gandara, J. Influence of pH and soil copper on adsorption of metalaxyl and penconazole by the surface layer of vineyard soils. *Journal of Agricultural and Food Chemistry* **2006**, *54*, 8155-8162.

Asthagiri, A.; Hazen, R. M. An ab inito study of adsorption of alanine on the chiral calcite surface. *Molecular Simulation* **2007**, *33*, 343-351.

Berthod, A. Chiral recognition mechanisms. *Analytical Chemistry* **2006**, *78*, 2093-2099.

Bondy, S. C.; Harrington, M. E. L-Amino acids and D-glucose bind stereoselectively to a colloidal clay. *Science* **1979**, *203*, 1243-1244.

Bonner, W. A.; Kavasmaneck, P. R.; Martin, F. S. Flores, J. T. Asymmetric adsorption of alanine by quartz. *Science* **1974**, *186*, 143-144.

Buerge, I. J.; Poiger, T.; Muller, M. D.; Buser, H. –R. Enantioselective degradation of metalaxyl in soils: Chiral preference changes with soil pH. *Environmental Science and Technology* **2003**, *37*, 2668-2674.

Buser, H. –R.; Muller, M. D.; Poiger, T.; Balmer, M. E. Environmental behavior of the chiral acetamide pesticide metalaxyl: Enantioselective degradation and chiral stability in soil. *Environmental Science and Technology* **2002**, *36*, 221-226.

Chen, S.; Liu, W. Enantioselective degradation of metalaxyl in anaerobic activated sewage sludge. *Bulletin of Environmental Contamination and Toxicology* **2009**, *82*, 327-331.

De Wilde, T.; Mertens, J.; Spanoghe, P.; Ryckeboer, J.; Jaeken, P.; Springael, D. Sorption kinetics and its effects on retention and leaching. *Chemosphere* **2008**, *72*, 509-516.

Ellington, J. J.; Evans, J. J.; Prickett, K. B.; Champion Jr., W. L. High performance liquid chromatographic separation of the enantiomers of organophosphorus pesticides on polysaccharide chiral stationary phases. *Journal of Chromatography A* **2001**, *928*, 145-154.

Fernandes, M. C.; Cox, L.; Hermosin, M. C. Cornejo, J. Adsorption—desorption of metalaxyl as affecting dissipation and leaching in soils: Role of mineral and organic components. *Pest Management Science* **2003**, *59*, 545-552.

Fernandes, M. C. Cox, L.; Hermosin, M. C.; Cornejo, J. Organic amendments affecting adsorption, leaching and dissipation of fungicides in soils. *Pest Management Science* **2006**, *62*, 1207-1215.

Giles, C. H.; Smith, D.; Huitson, A. A general treatment and classification of the solute adsorption isotherm. *Journal of Colloid and Interface Science* **1974**, *47*, 755-765.

Han, J. W.; Sholl, D. S. Enantiospecific adsorption of amino acids on hydroxylated quartz (0001). *Langmuir* **2009**, *25*, 10737-10745.

Han, J.W.; Sholl, D. S. Enantiospecific adsorption of amino acids on hydroxylated quartz (10$\bar{1}$0). *Physical Chemistry Chemical Physics* **2010**, *12*, 8024-8032.

Hazen, R. M.; Filley, T. R.; Goodfriend, G. A. Selective adsorption of L- and D-amino acids on calcite: Implications for biochemical homochirality. *PNAS* **2001**, *98*, 5487-5490.

Hazen, R. M.; Sholl, D. S. Chiral selection on inorganic crystalline surfaces. *Nature* **2003**, *2*, 367-374.

Horvath, J. D.; Gellman, A. J. Naturally chiral surfaces. *Topics in Catalysis* **2003**, *25*, 9-15.

Jarman, J. L.; Jones, W. J.; Howell, L. A.; Garrison, A. W. Application of capillary electrophoresis to study the enantioselective transformation of five chiral pesticides in aerobic soil slurries. *Journal of Agricultural and Food Chemistry* **2005**, *53*, 6175-6182.

Julg, A.; Ozias, Y. Asymmetric adsorption of ethyliminium cation on kaolinite and L-homochirality of amino acids in proteins. *Journal of Molecular Structure* (*Theochem*) **1988**, *179*, 17-25.

Li, Z.; Wu, T.; Li, Q.; Zhang, B.; Wang, W.; Li, J. Characterization of racemization of chiral pesticides in organic solvents and water. *Journal of Chromatography A* **2010**, *1217*, 5718-5723.

Marucchini, C.; Zadra, C. Stereoselective degradation of metalaxyl and metalaxyl-m in soil and sunflower plants. *Chirality* **2002**, *14*, 32-38.

Marin-Benito, J. M.; Sanchez-Martin, M. J.; Andrades, M. S.; Perez-Clavijo, M.; Rodriguez-Cruz, M. S. Effect of spent mushroom substrate amendment of vineyard soils on the behavior of fungicides: 1. Adsorption—Desorption of penconazole and metalaxyl by soils and subsoils. *Journal of Agricultural and Food Chemistry* **2009**, *57*, 9634-9642.

Matallo, M. Romero, E. Sanchez-Rasero, F. Adsorption of mecoprop and dichlorprop on calcerous and organic matter amended soils. *Journal of Environmental Science and Health, Part B: Pesticides Food Contaminants and Agricultural Wastes* **1998**, *33*, 51-66.

Messina, A.; Sinibaldi, M. CEC enantioseparations on chiral monolithic columns: A study of the stereoselective degradation of (*R*/*S*)-dichlorprop [2-(2,4-dichlorophenoxy) propionic acid] in soil. *Electrophoresis* **2007**, *28*, 2613-2618.

Monkiedje, A.; Spiteller, M. Sorptive behavior of the phenylamide fungicides mefenoxam and metalaxyl, and their acid metabolite in typical Cameroonian and German soils. *Chemosphere* **2002**, *49*, 659-668.

Monkiedje, A.; Spiteller, M.; Bester, K. Degradation of racemic enantiopure metalaxyl in tropical and temperate soils. *Environmental Science and Technology* **2003**, *37*, 707-712.

Monkiedje, A.; Zuehlke, S.; Maniepi, S. J. N.; Spiteller, M. Elimination of racemic and enantioenriched metalaxyl based fungicides under tropical conditions in the field. *Chemosphere* **2007**, *69*, 655-663.

Muller, M. D.; Buser, H. –R. Environmental behavior of acetamide pesticide stereoisomers. 2. Stereo- and enantioselective degradation in sewage sludge and soil. *Environmental Science and Technology* **1995**, *29*, 2031-2037.

Muller, M. D.; Buser, H. –R. Conversion reactions of various phenoxyalkanoic herbicides in soil. 1. Enantiomerization and enantioselective degradation of the chiral 2-phenoxypropionic acid herbicides. *Environmental Science and Technology* **1997**, *31*, 1953-1959.

Muller, T. A.; Kohler, H.-P.E. Chirality of pollutants—effects on metabolism and fate. *Applied Microbiology and Biotechnology* **2004**, *64*, 300-316.

Nillos, M. G.; Qin, S.; Larive, C. Schlenk, D.; Gan, J. Epimerization of cypermethrin stereoisomers in alcohols. *Journal of Agricultural and Food Chemistry* **2009**, *57*, 6938-6943.

Oravec, M.; Simek, Z.; Holoubek, I. The effect of humic acid and ash on enantiomeric fraction change of chiral pollutants. *Colloids and Surfaces A: Physiochemical Engineering Aspects* **2010**, *359*, 60-65.

Qin, S.; Gan, J. Abiotic enantiomerization of permethrin and cypermethrin: Effects of organic solvents. *Journal of Agricultural and Food Chemistry* **2007**, *55*, 5734-5739.

Reist, M.; Testa, B.; Carrupt, P. A.; Jung, M.; Schurig, V. Racemization, enantiomerization, diastereomerization, and epimerization: Their meaning and pharmacological significance. *Chirality* **1995**, *7*, 396-400.

Rodriguez-Cruz, M. S.; Sanchez-Martin, M. J.; Andrades, M. S.; Sanchez-Camazano, M. Comparison of pesticide sorption by physicochemically modified soils with natural soils as a function of soil properties and pesticide hydrophobicity. *Soil and Sediment Contamination* **2006**, *15*, 401-415.

Romero, E.; Matallo, M. B.; Pena, A.; Sanchez-Rasero, F.; Schmitt-Kopplin, Ph.; Dios, G. Dissipation of racemic mecoprop and dichlorprop and their pure *R*-enantiomers in three calcareous soils with and without peat addition. *Environmental Pollution* **2001**, *111*, 209-215.

Sanchez-Martin, M. J.; Rodriguez-Cruz, M. S.; Andrades, M. S.; Sanchez-Camazano, M. Efficiency of different clay minerals modified with a cationic surfactant in the adsorption of pesticides: Influence of clay type and pesticide hydrophobicity. *Applied Clay Science* **2006**, *31*, 216-228.

Saha, T.; Sukul, P. Metalaxyl—its persistence and metabolism in soil. *Toxicological and Environmental Chemistry* **1997**, *58*, 251-258.

Sharma, D.; Awasthi, M. D. Adsorption and movement of metalaxyl in soils under unsaturated flow conditions. *Plant and Soil* **1997**, *195*, 293-298.

Sharom, M. S.; Edgington, L. V. The adsorption, mobility, and persistence of metalaxyl in soil and aqueous systems. *Canadian Journal of Plant Pathology* **1982**, *4*, 334-340.

Siffert, B.; Naidja, A. Stereoselectivity of montmorillonite in the adsorption and deamination of some amino acids. *Clay Minerals* **1992**, *27*, 109-118.

Sukop, M.; Cogger, C. G. Adsorption of carbofuran, metalaxyl, and simazine: Koc evaluation and relation to soil transport. *Journal of Environmental Science and Health Part B: Pesticides, Food Contaminants, and Agricultural Wastes* **1992**, 27, 565-590.

Sukul, P.; Spiteller, M. Metalaxyl: Persistence, degradation, metabolism, and analytical methods. *Reviews of Environmental Contamination and Toxicology* **2000a**, *164*, 1-26.

Sukul, P.; Spiteller, M. Sorption study on metalaxyl in soils of different physiochemical properties. *Fresenius Environmental Bulletin* **2000b**, *9*, 701-710.

Sukul, P.; Spiteller, M. Influence of biotic and abiotic factors on dissipating metalaxyl in soil. *Chemosphere* **2001**, *45*, 941-947.

Sun, M; Liu, D.; Zhou, G.; Li, J.; Qiu, X.; Zhou, Z.; Wang, P. Enantioselective degradation and chiral stability of malathion in environmental samples. *Journal of Agricultural and Food Chemistry* **2012**, *60*, 372-379.

Wang, P.; Jiang, S. R.; Qiu, J. Wang, Q. X.; Wang, P.; Zhou, Z. Q. Stereoselective degradation of ethofumesate in turfgrass and soil. *Pesticide Biochemistry and Physiology* **2005**, *82*, 197-204.

Wedyan, M.; Preston, M. R. Isomer-selective adsorption of amino acids by components of natural sediments. *Environmental Science and Technology* **2005**, *39*, 2115-2119.

Wolf, C. *Dynamic Stereochemistry of Chiral Compounds-Principles and Applications* **2008**, The Royal Society of Chemistry, Cambridge, United Kingdom.

Zipper, C.; Suter, M, J.; Haderlein, S. B.; Gruhl, M.; Kohler, H. P. Changes in the enantiomeric ratio of (*R*)- to (*S*)-mecoprop indicate in situ biodegradation of this chiral herbicide in a polluted aquifer. *Environmental Science and Technology* **1998**, *32*, 2070-2076.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Sorption and enantiomerization of the current use chiral pesticides malathion and metalaxyl to mineral surfaces was investigated. This research is unique in using chiral analysis coupled with achiral analysis to investigate sorption of the racemate and individual enantiomers of malathion and metalaxyl. The main conclusions are presented below:

1. Sorption of malathion racemate was investigated using HPLC analysis, which is considered a more specific and robust method of analysis compared to spectrophotometry analysis alone. Racemic malathion significantly sorbed to relatively pure minerals, implying the possibility of sorption to whole soils and sediments. Sorption of malathion can reduce its bioavailability, and hinder microbial degradation, enhancing persistence in the environment. Due to the negligible sorption to kaolinite, soils containing high amounts of kaolinite could cause leaching of malathion. Chiral analysis proved that sorption was nonenantioselective, with racemic enantiomeric fractions for all sorbents at all concentrations. However, chiral sorption for other pesticides should not be ruled out as a possibility, given the chirality of pesticides and the variety of chiral sorbents in the environment.

2. Sorption of individual malathion enantiomers to mineral surfaces showed complete enantiomerization to racemic of both malathion enantiomers when in contact with all sorbents studied. Enantiomerization of malathion reduces the efficacy of a chiral switch, implying that independent of the starting enantiomer, malathion will completely enantiomerize to the racemic form when in the presence of minerals. Enantiomerization was observed in water and β-cyclodextrin, but it was not complete, indicating that malathion-sorbent interactions resulted in more rapid enantiomerization. It has been hypothesized that malathion enantiomerization occurs in protic environments, where abstraction and subsequent addition of a proton at the chiral carbon is facilitating enantiomerization. Due to its hydrophobicity, malathion would more likely be sorbed to solids rather than in the aqueous phase in the environment. Malathion will racemize rapidly and completely when in contact with solids, rather than partial enantiomerization in the aqueous phase, thus negating the possibility of a chiral switch being used effectively for malathion. In addition, enantiomerization of malathion indicates that not all chiral pesticides can be marketed as single enantiomers. Prior to use and registration of single enantiomer pesticides, the behavior of these pesticides should be studied and possible enantiomerization in the environment should be considered.

3. Metalaxyl racemate and individual enantiomers were found to sorb significantly to bentonite and montmorillonite, but sorption was not observed for calcite or kaolinite. This implies the reduction of bioavailability and the subsequent persistence of metalaxyl

when in the presence of high capacity smectite clays. The opposite can be true as well; due to the low sorption of metalaxyl to calcite and kaolinite, and the moderate water solubility, the presence of these two minerals in soils could facilitate leaching of metalaxyl. Racemic EFs implying enantioselective sorption for metalaxyl was not observed. However, changes in EF for metalaxyl-M indicate that sorption was either enantioselective, or enantiomerization occurred in the presence of minerals. Since sorption of racemic metalaxyl was significantly lower than for metalaxyl-M, it can be hypothesized that selective sorption of *R*-(-)-metalaxyl, the main component of metalaxyl-M, is occurring. On the contrary, EFs changed for calcite and kaolinite, where sorption was negligible; thereby inferring that enantiomerization is taking place instead of enantioselective sorption.

Malathion and metalaxyl sorption to environmental surfaces has been reported in the literature, no sorption research has been done with the individual enantiomers by chiral analysis. This research provides insight into the sorption activities of malathion and metalaxyl enantiomers, which indicate complete enantiomerization to racemic for malathion and partial enantiomerization or enantioselective sorption for metalaxyl. This research presents the first evidence of enantiomerization of a chiral pesticide during sorption experiments and explores the possibility of enantioselective sorption.

Recommendations for Future Work

Malathion is a widely used chiral pesticide that is lacking in sorption and enantioselective degradation research. As this research suggests, malathion enantiomers undergo enantiomerization independent of the starting enantiomer or mineral surface. More research is recommended using chiral analysis of sorption and degradation processes of malathion in environmental matrices such as whole soils and sediments. Since the presence of an unknown peak appeared to indicate degradation of malathion in the presence of water and minerals, mass spectrometry should be utilized to identify the peak and determine the possible degradation mechanisms.

Metalaxyl has been widely studied for sorption of the racemate by achiral analysis, but the current formulation of metalaxyl-M, which is the most common form used in the environment, has not. More research is recommended using metalaxyl-M and pure *R*-(-)- and *S*-(*+*)-metalaxyl in sorption experiments to detect possible enantiomerization and to determine if enantioselective sorption occurs. Since *R*-(-)-metalaxyl is the enantiomer with pesticidal activity, application of the *R*-(-)-enantiomer and subsequent enantiomerization to the relatively inactive *S*-(*+*)-enantiomer could cause issues with the dose effectiveness of metalaxyl-M in the environment. If enantioselective sorption occurs for *R*-(-)-metalaxyl, this could impact its bioavailability and in turn its persistence in the environment.

Soils and sediments contain organic matter which is considered the most important factor in the sorption of non-ionic pesticides. Sorption by natural organic

matter (NOM), including humic acids, should be investigated for malathion and metalaxyl since NOM and humic acids could possibly contain several stereogenic centers for enantioselective sorption to occur. In the absence of organic matter, or in limited amounts, the presence and content of clay minerals may significantly influence the sorption of non-ionic pesticides such as malathion and metalaxyl. Therefore, it is important to study the sorption of pesticides to clay minerals as well as NOM and humic acids to gain insight to the pesticide sorption of complex matrices such as soils and sediments.

Future work using radiolabeled isotopes could help understand the enantiomerization mechanisms in the presence of minerals for malathion and metalaxyl. Deuterium would be beneficial in understanding whether the removal of the proton on the chiral center is the cause of enantiomerization facilitated by the sorbent. Conducting experiments in D_2O would also be beneficial in understanding whether the proton is being extracted from the solvent or the sorbent. Although malathion and metalaxyl are non-ionic pesticides, pH could be a factor in affecting sorption and enantiomerization. At environmental pH, organic matter is negatively charged, while clay minerals can display both cationic and anionic sites. Metalaxyl can sorb on environmental surfaces via hydrogen bonding of the amine or ester groups, which can be a function of pH. Enantioselective degradation studies have shown that metalaxyl enantioselectivites can vary as a function of pH, even though metalaxyl is a non-ionic pesticide. The enantiomerization mechanisms proposed for malathion and metalaxyl in

this dissertation involves the active chiral proton and would be a function of pH. To understand the effect of pH, future work is recommended for monitoring and modifying the pH to determine its role in sorption and enantiomerization of malathion and metalaxyl.

APPENDICES

Appendix A

Supplementary Material for Chapter 2

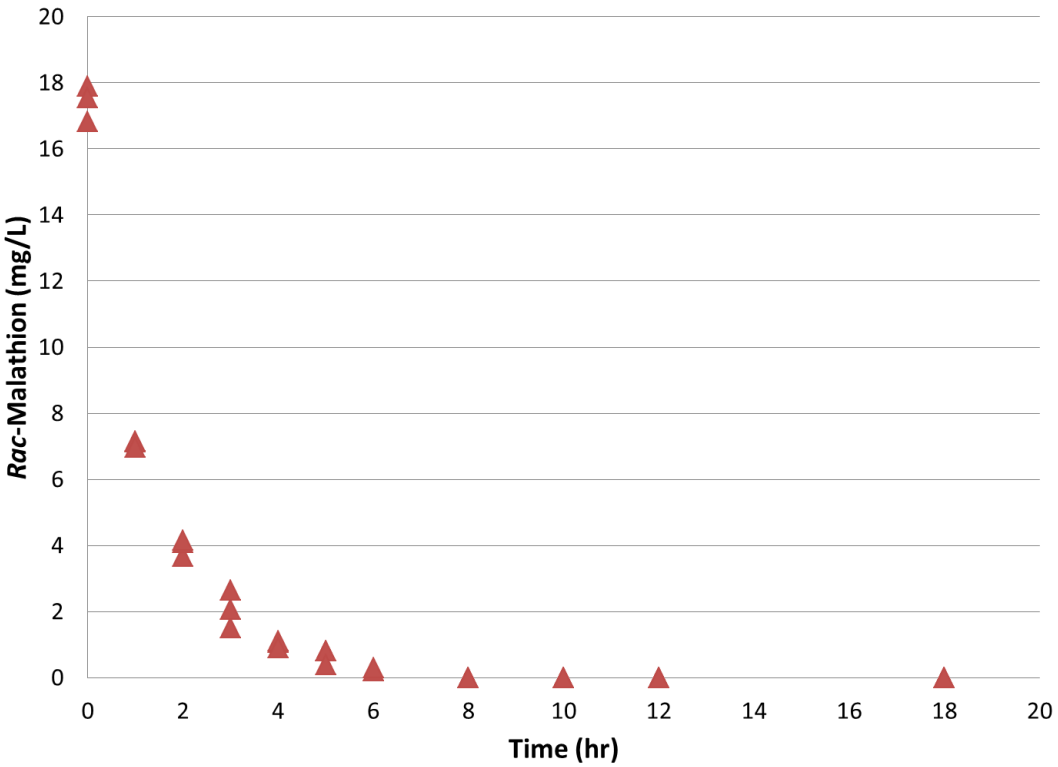


Figure A.1: Concentration of *rac*-malathion in the aqueous phase versus time after contact with 50 mg of bentonite.

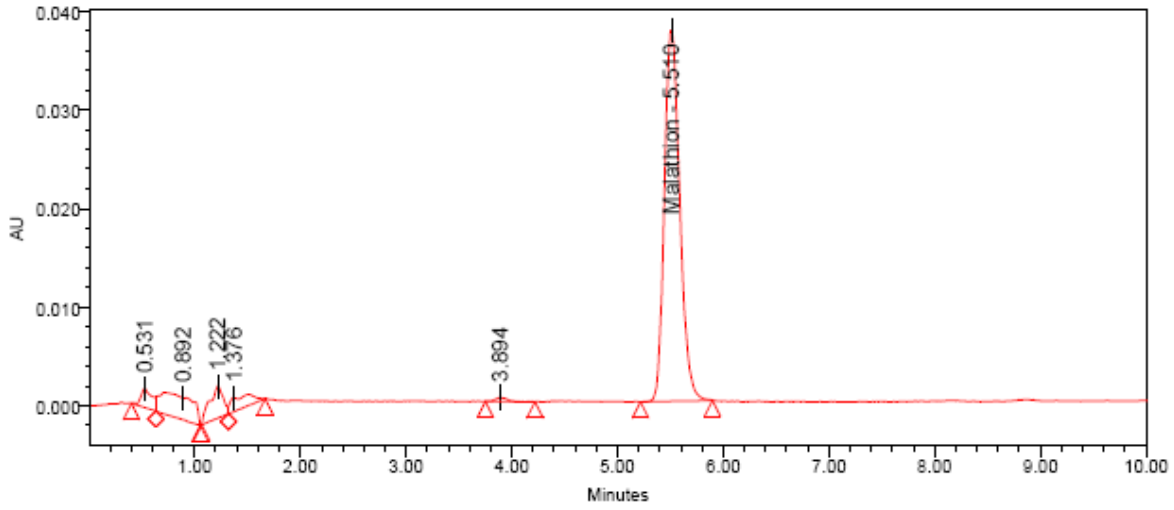


Figure A.2: Achiral HPLC chromatogram of malathion.

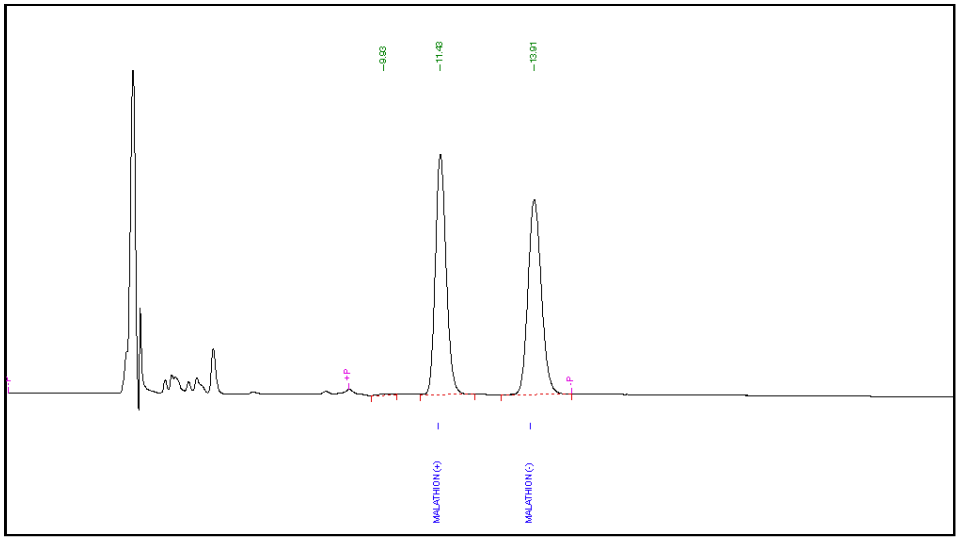


Figure A.3: Chiral HPLC chromatogram of *rac*-malathion. *R*-(*+*)-malathion elutes first, followed by *S*-(-)-malathion.

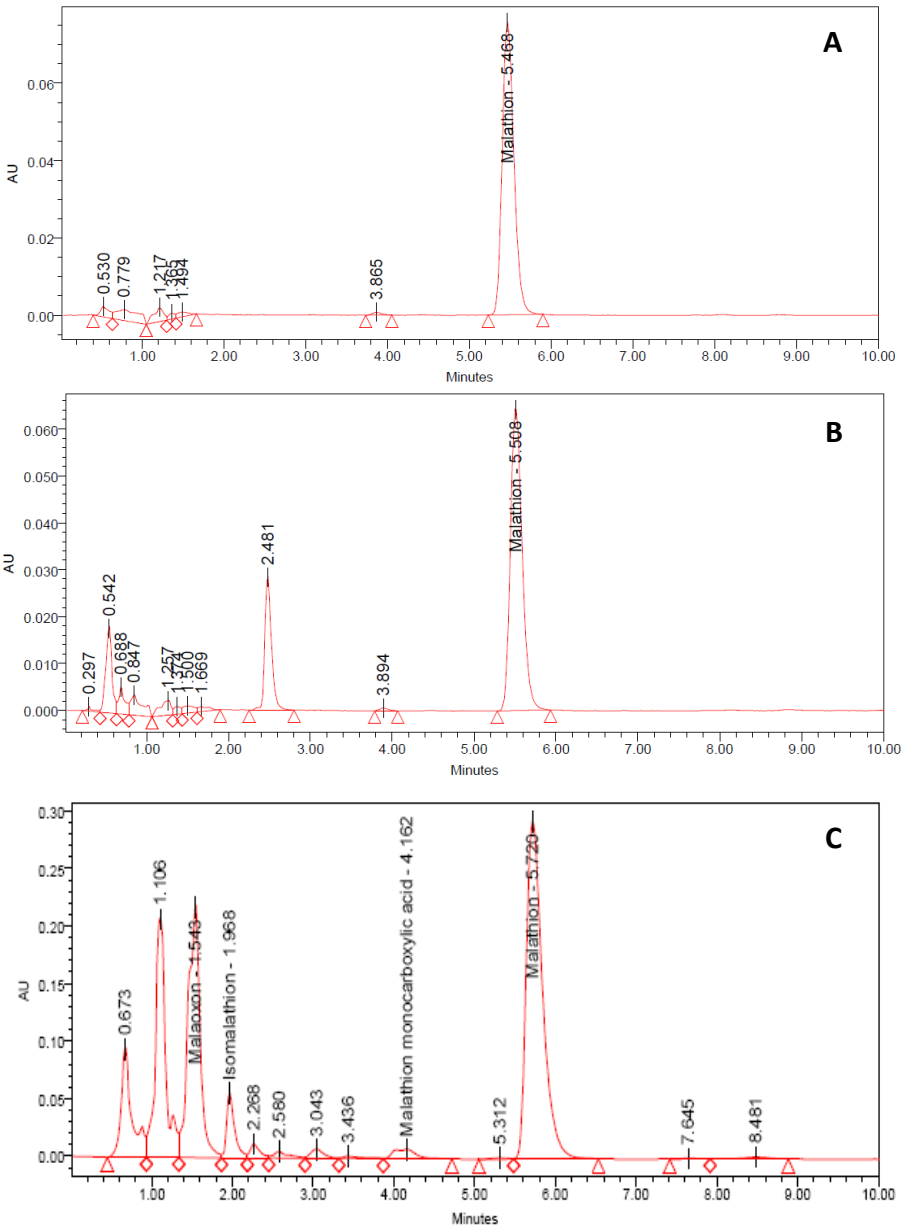
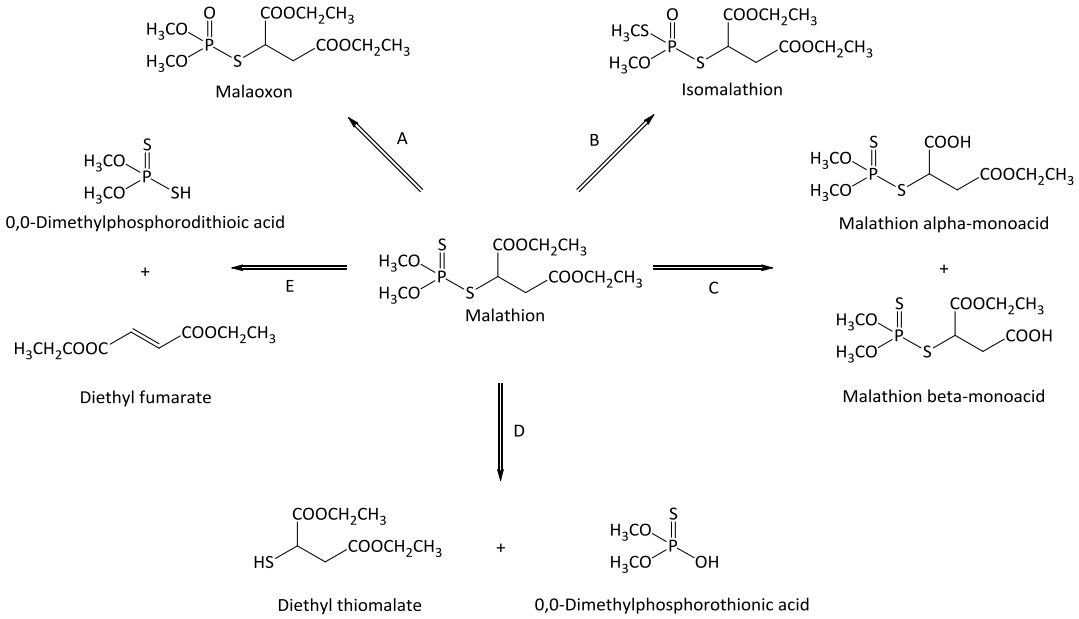


Figure A.4: HPLC achiral chromatograms of *rac*-malathion standard **A**) before and **B**) after two hours incubation and **C**) potential breakdown products malaoxon, isomalathion, and malathion monocarboxylic acid. The presence of an unknown peak at 2.5 min in **B** indicates the malathion is possibly breaking down in water, but is not identified as one of the degradation products in **C**.



A: Oxidation
B: Thermal or photochemical isomerization
C: Carboxyl ester hydrolysis
D: Phosphorus-sulfur cleavage by water or hydroxide
E: Sulfur-carbon elmination

Figure A.5: Potential breakdown products of malathion in water. Adapted from Wolfe et al. (1975).

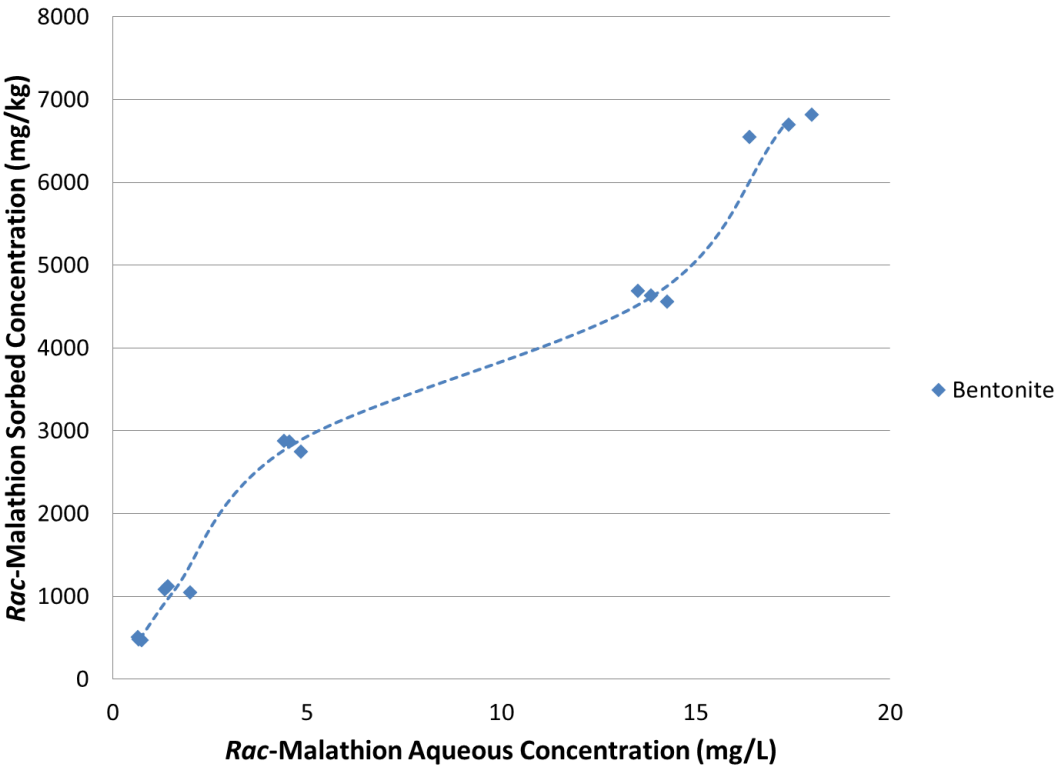


Figure A.6: Bentonite sorption isotherm of r*ac*-malathion. Dotted line demonstrates isotherm shape and does not represent a model fit.

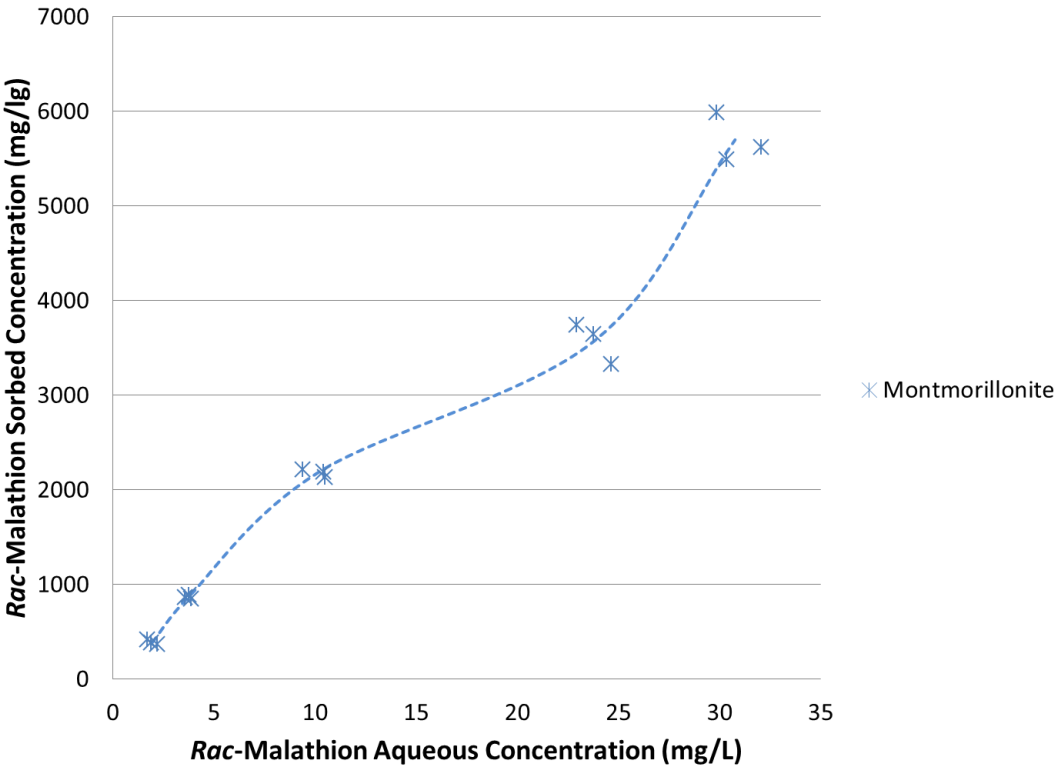


Figure A.7: Montmorillonite sorption isotherm of r*ac*-malathion. Dotted line demonstrates isotherm shape and does not represent a model fit.

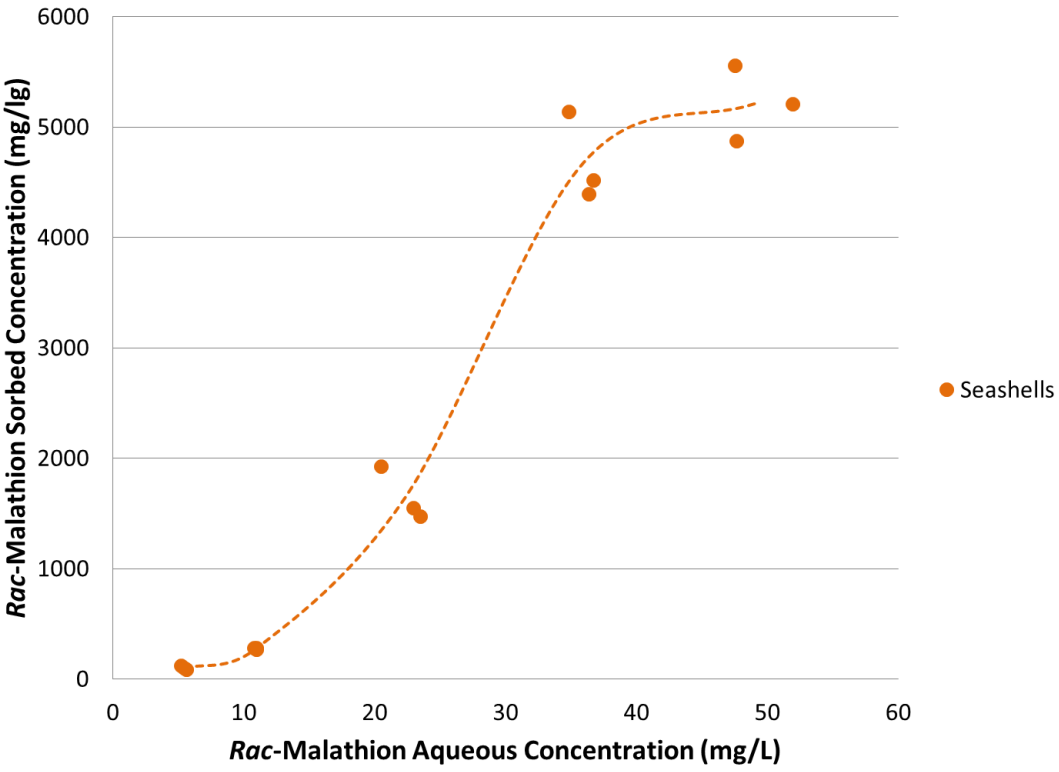


Figure A.8: Seashells sorption isotherm of r*ac*-malathion. Dotted line demonstrates isotherm shape and does not represent a model fit.

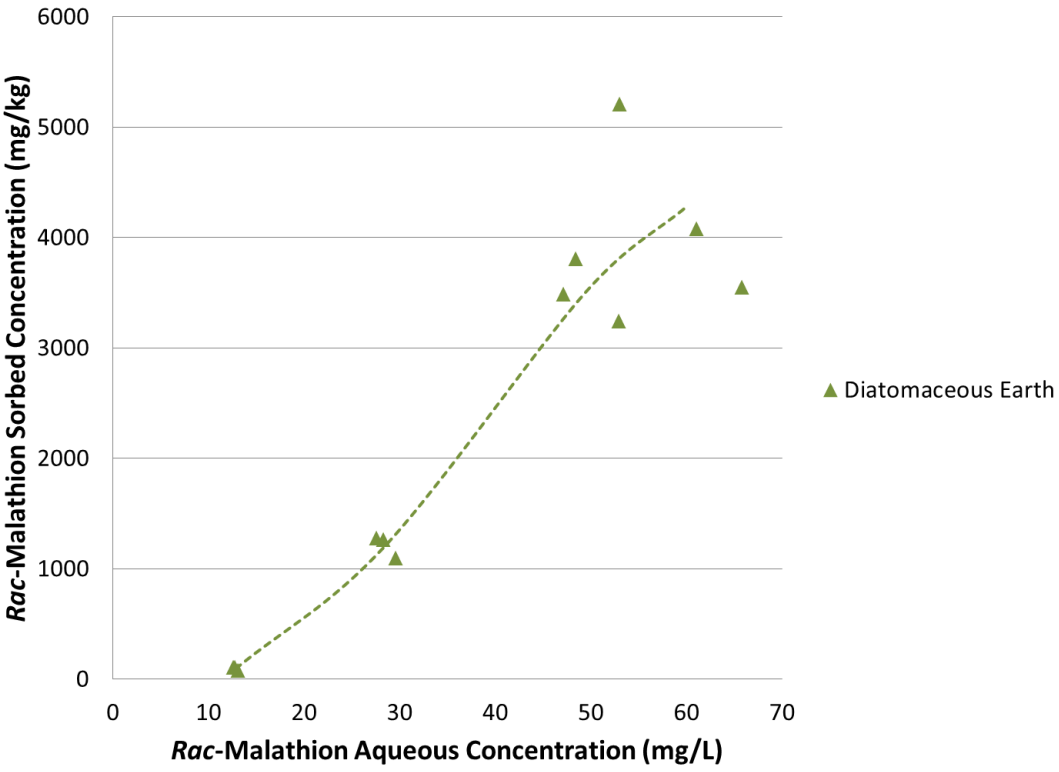


Figure A.9: Diatomaceous earth sorption isotherm of r*ac*-malathion. Dotted line demonstrates isotherm shape and does not represent a model fit.

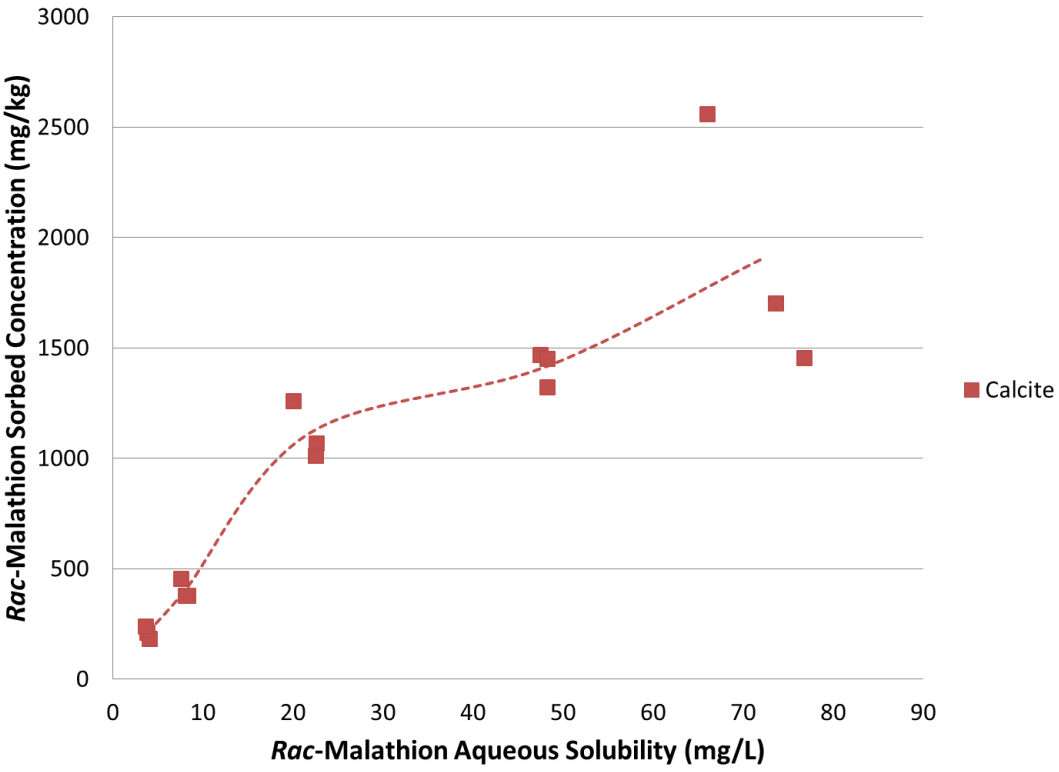


Figure A.10: Calcite sorption isotherm of r*ac*-malathion. Dotted line demonstrates isotherm shape and does not represent a model fit.

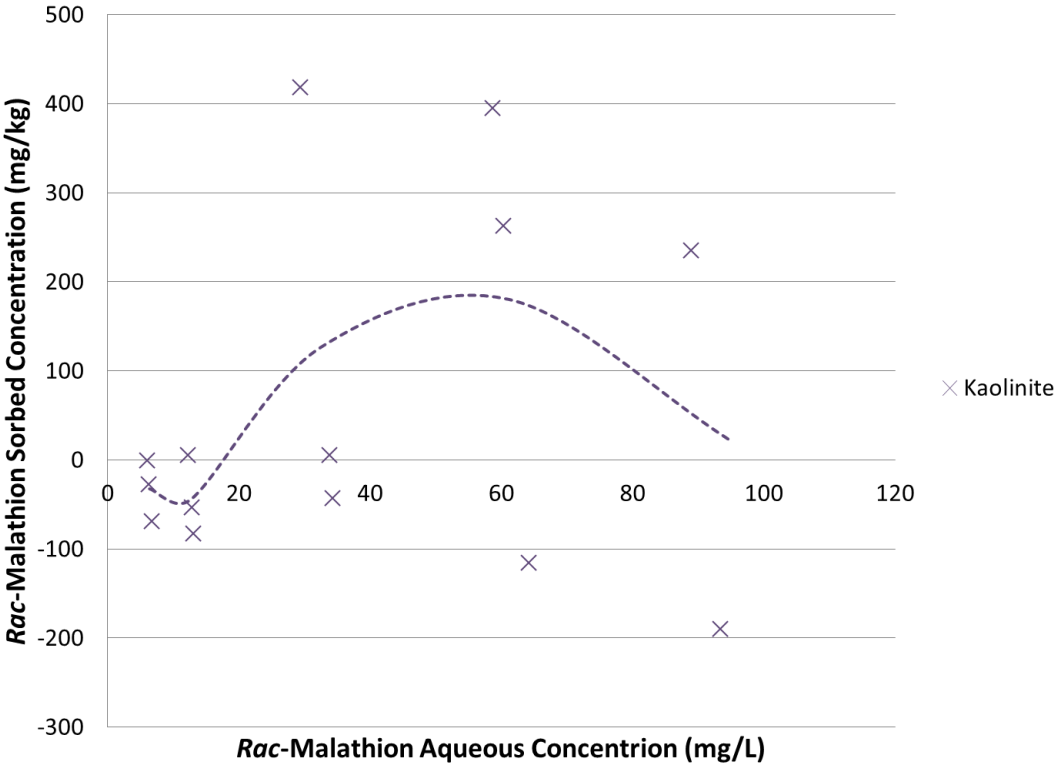


Figure A.11: Kaolinite sorption isotherm of r*ac*-malathion. Dotted line demonstrates isotherm shape and does not represent a model fit. Kaolinite sorption was negligible as observed by negative sorbed concentration values.

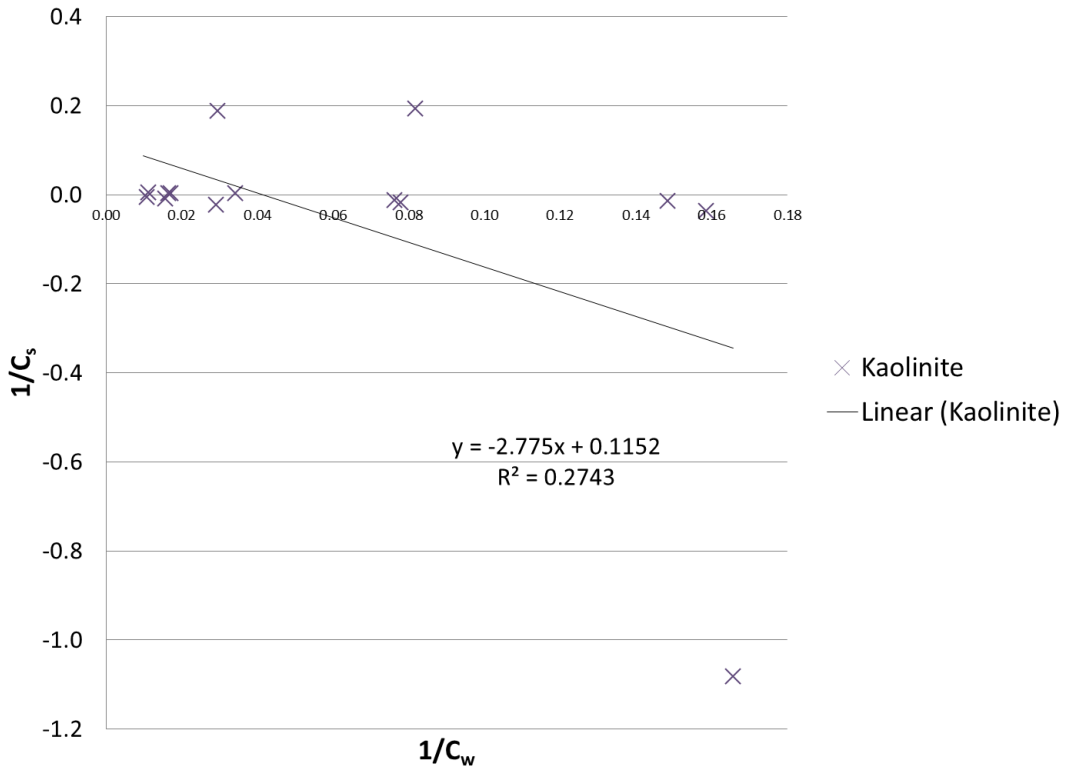


Figure A.12: Kaolinite Langmuir sorption isotherm of *rac*-malathion. C_s = *rac*-malathion sorbed concentration (mg/kg); C_w = *rac*-malathion aqueous concentration (mg/L) after two hours equilibration.

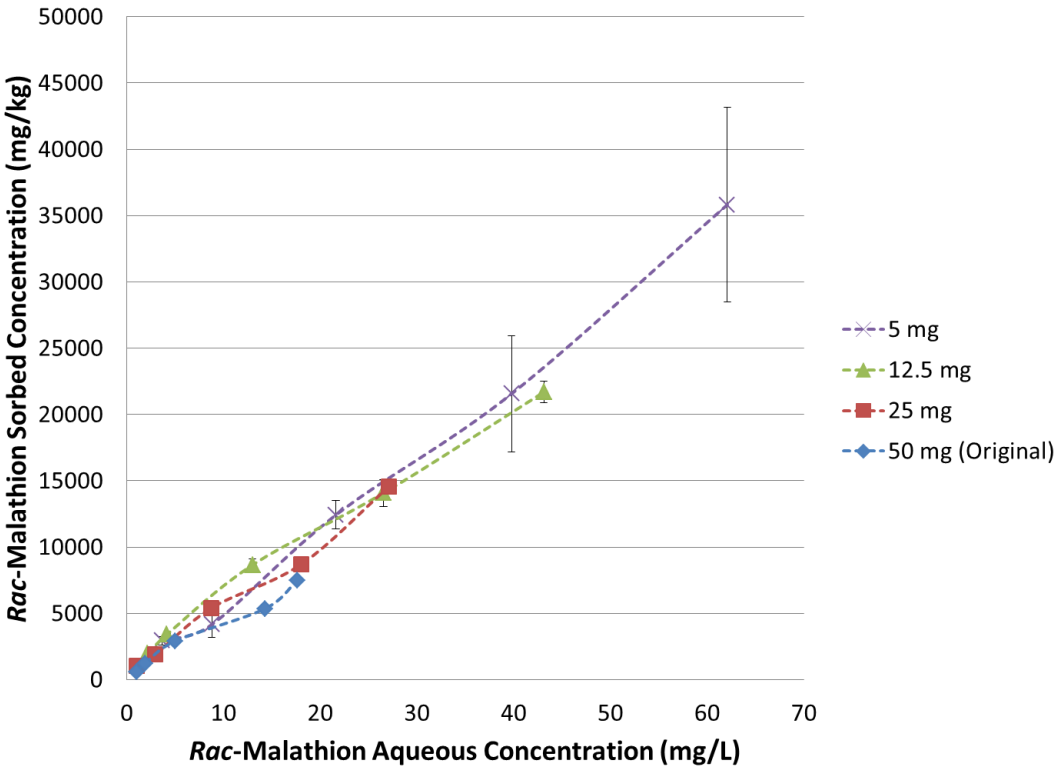


Figure A.13: Bentonite sorption isotherms of *rac*-malathion with modified sorbent weight. Dotted lines demonstrate isotherm shapes and do not represent model fits. Error bars represent standard deviations of triplicates.

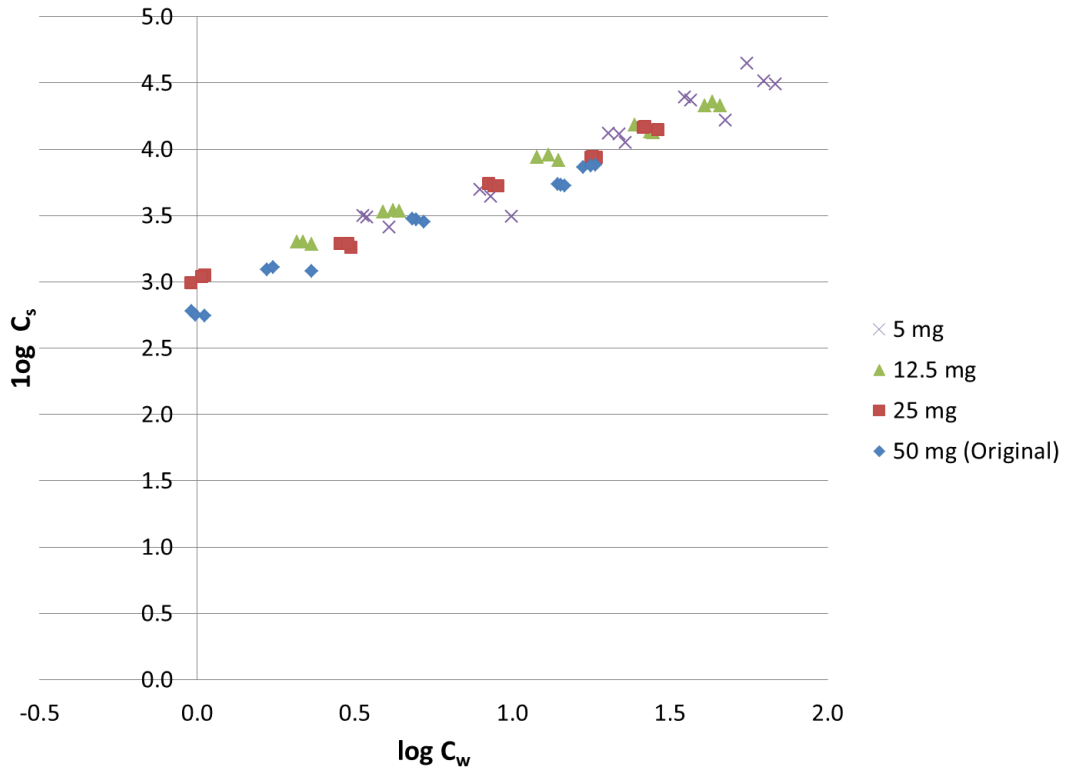


Figure A.14: Bentonite Freundlich sorption isotherms of *rac*-malathion with modified sorbent weight. C_s = *rac*-malathion sorbed concentration (mg/kg); C_w = *rac*-malathion aqueous concentration (mg/L) after two hours equilibration. Error bars represent standard deviations of triplicates.

Appendix B

Supplementary Material for Chapter 3

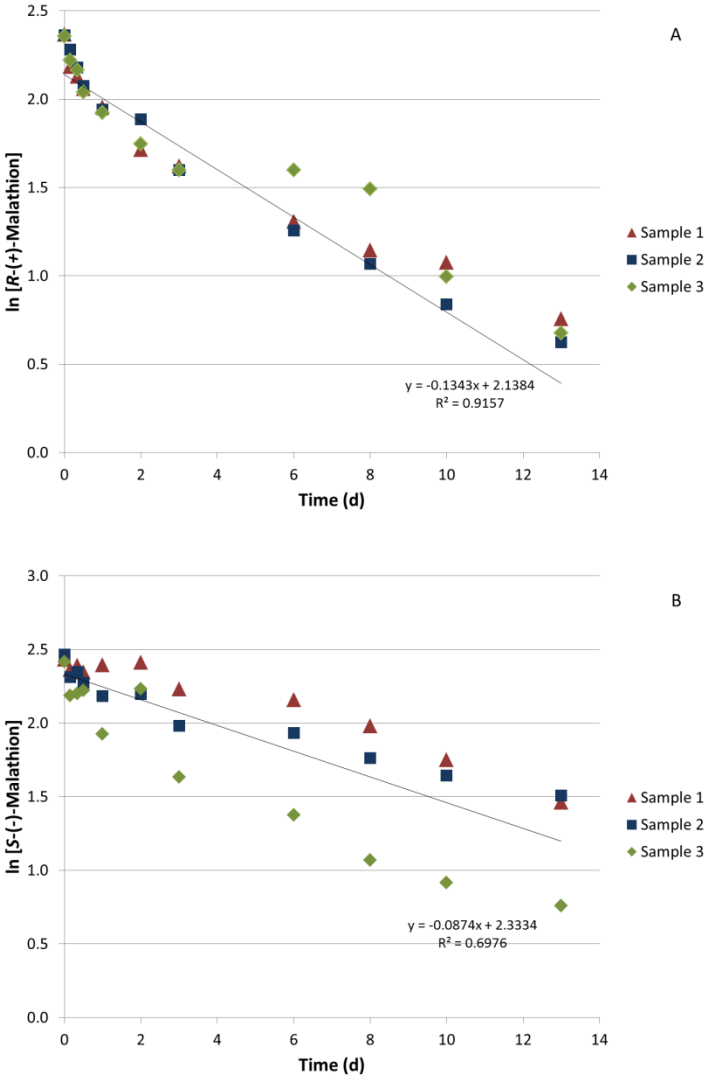


Figure B.1: First order degradation of **A**) *R*-(*+*)-malathion and **B**) *S*-(-)-malathion in water. Samples 1, 2, and 3 represent individual test tubes. The linear regression was performed on all data points.

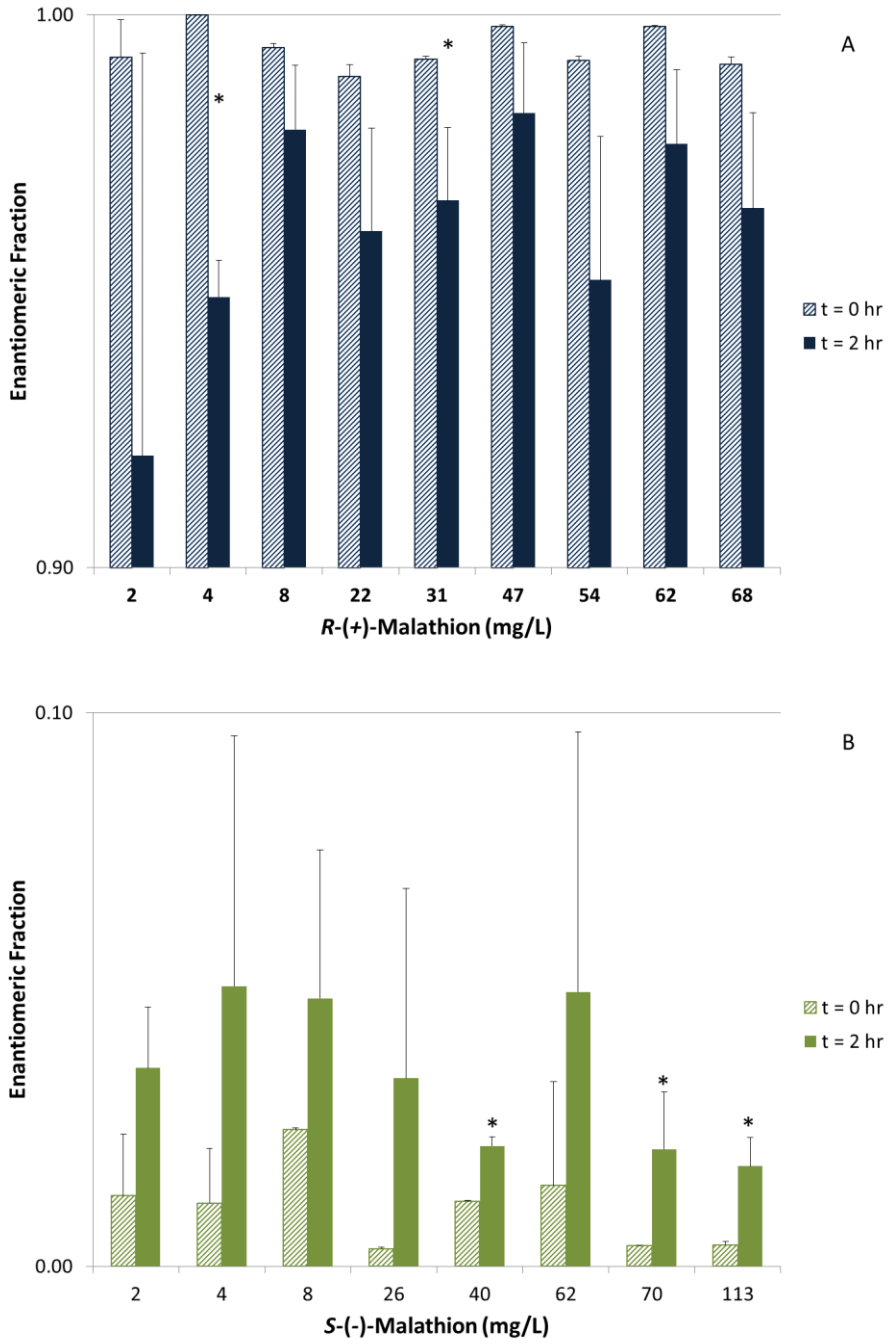


Figure B.2: Enantiomerization in water after two hours for **A**) *R*-(*+*)-malathion and **B**) *S*-(-)-malathion. The scales for both figures have been altered for data clarity. Error bars represent standard deviations of triplicates. A "*" indicates that the result is significantly different ($p < 0.05$).

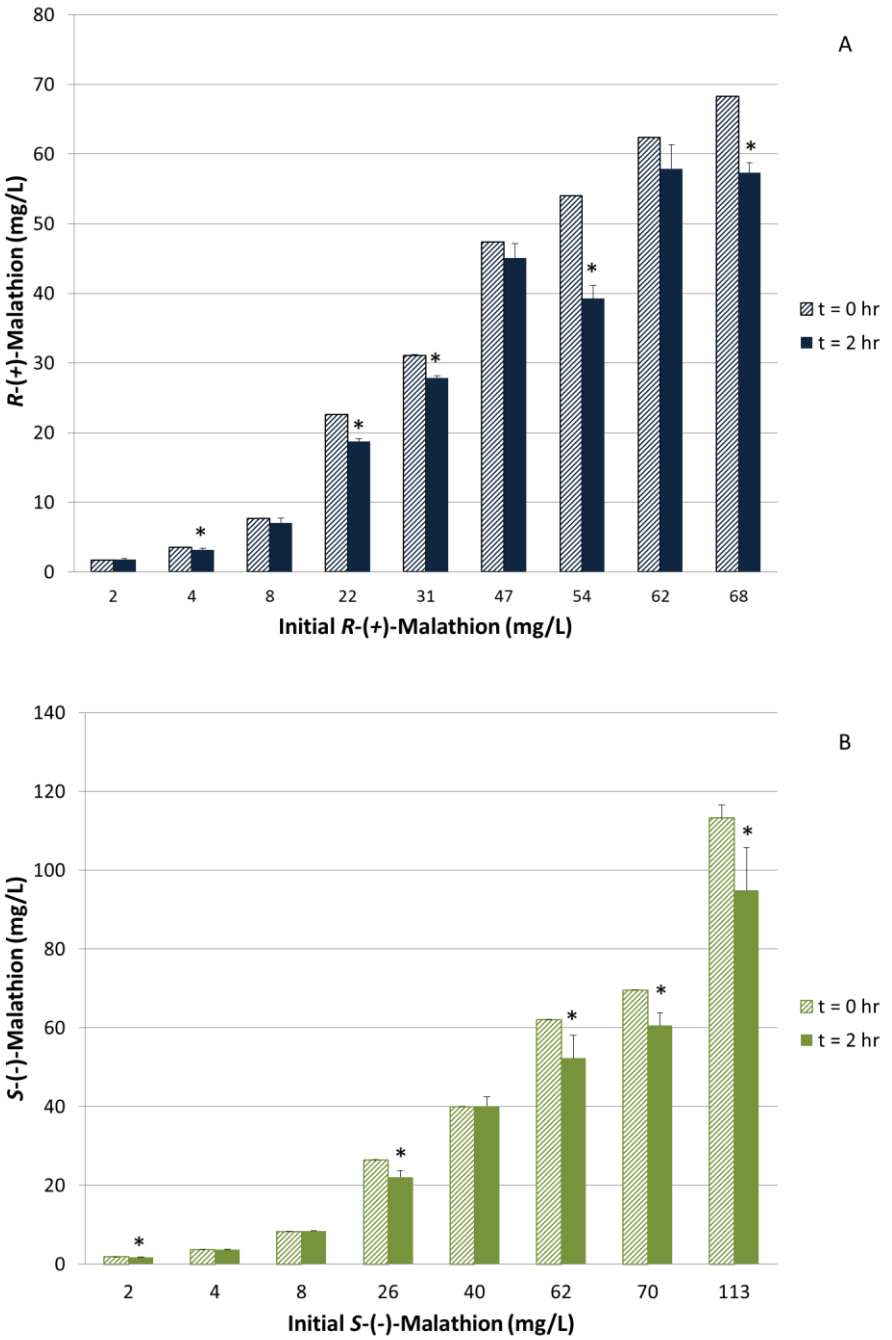


Figure B.3: Loss of **A**) *R*-(*+*)-malathion and **B**) *S*-(-)-malathion in water after two hours. Error bars represent standard deviations of triplicates. A "*" indicates the difference is statistically significant ($p < 0.05$).

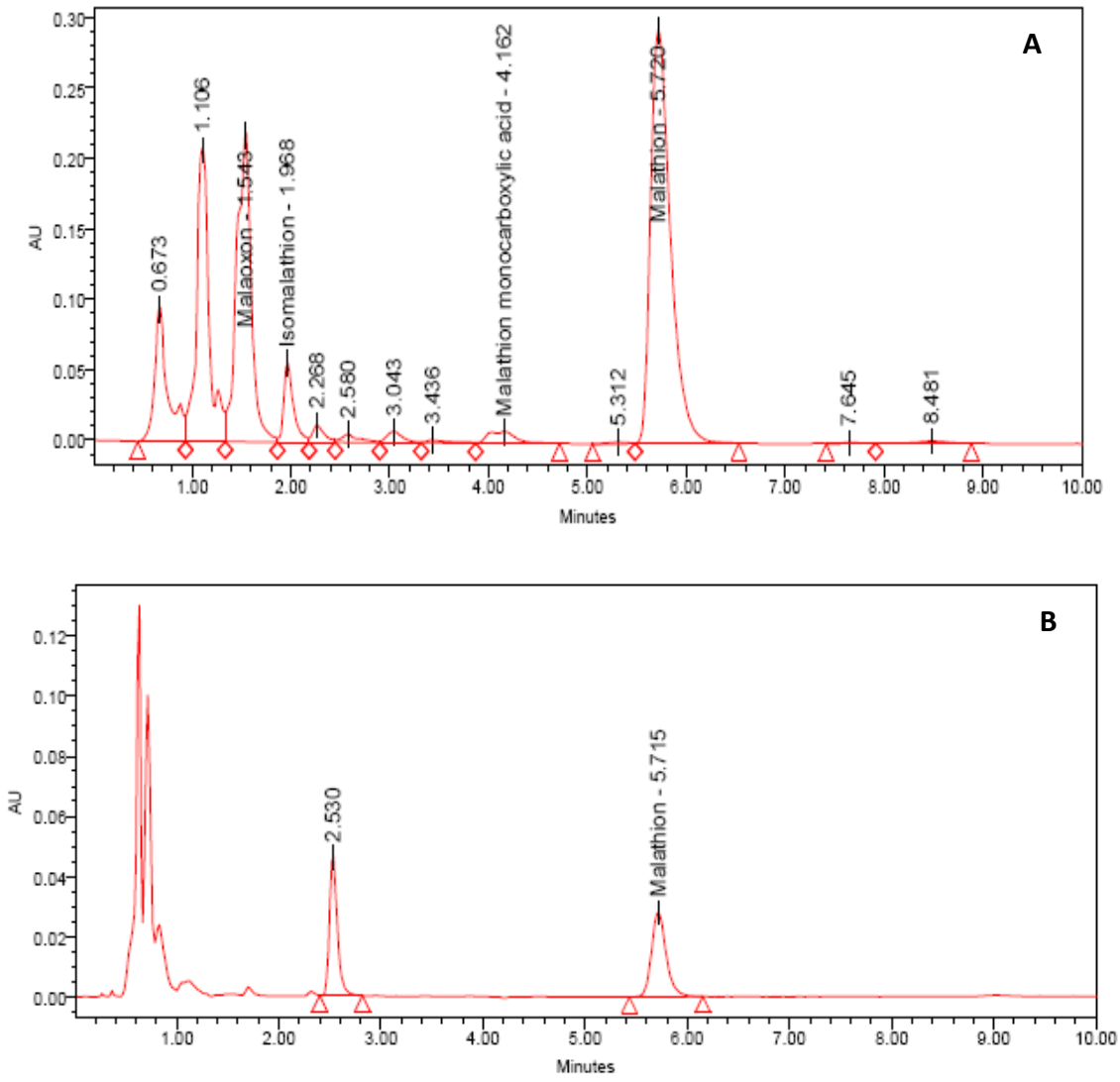


Figure B.4: HPLC achiral chromatograms of **A**) malathion and potential breakdown products malaoxon, isomalathion, and malathion monocarboxylic acid and **B**) *R*-(*+*)-malathion water control after two hours. The presence of an unknown peak at 2.5 min in **B** indicates the malathion is possibly breaking down in water, but is not identified as one of the degradation products in **A**.

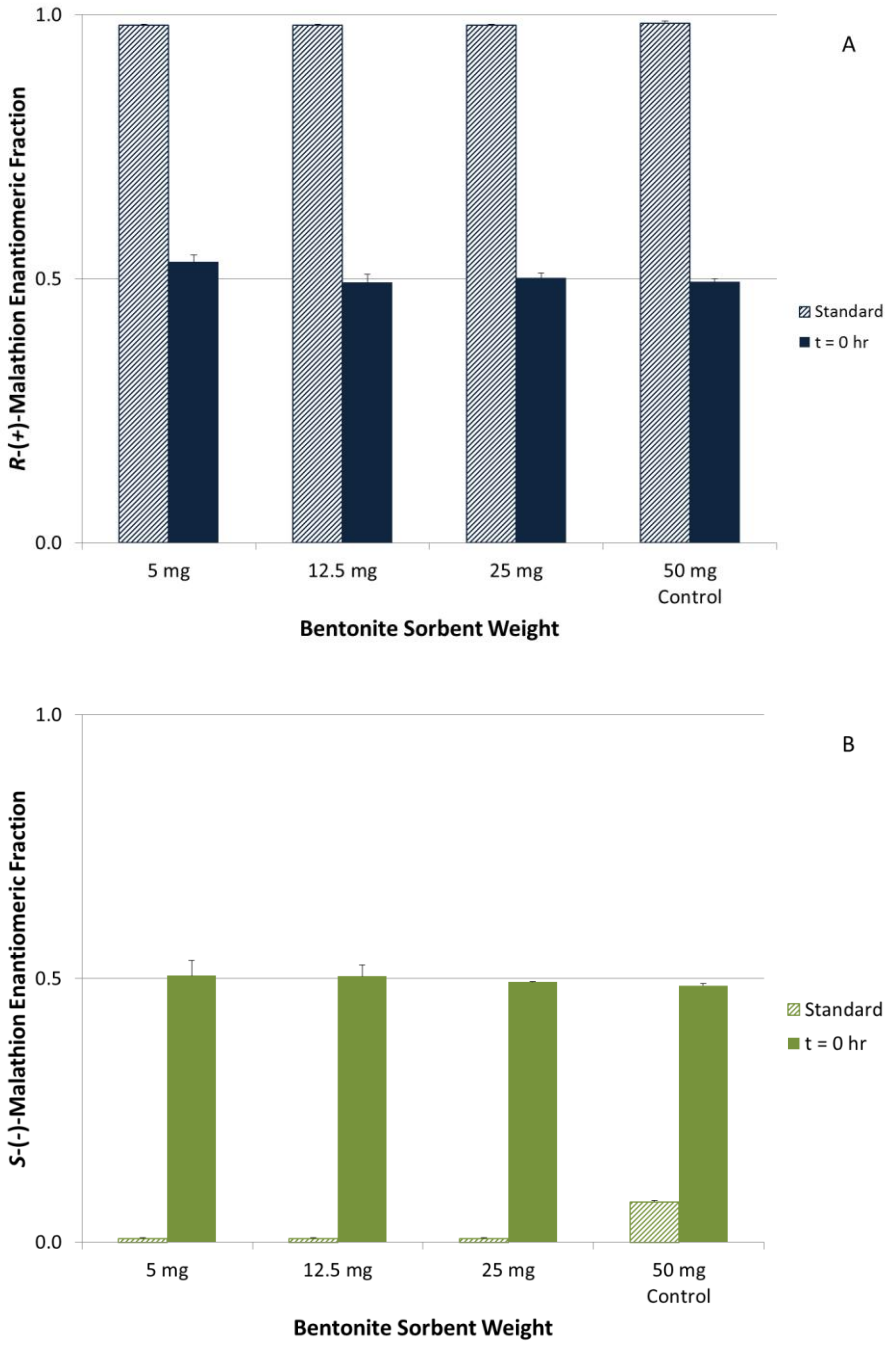


Figure B.5: Enantiomerization of **A**) *R*-(*+*)-malathion and **B**) *S*-(-)-malathion after immediate contact with bentonite. Error bars represent standard deviations of triplicates.

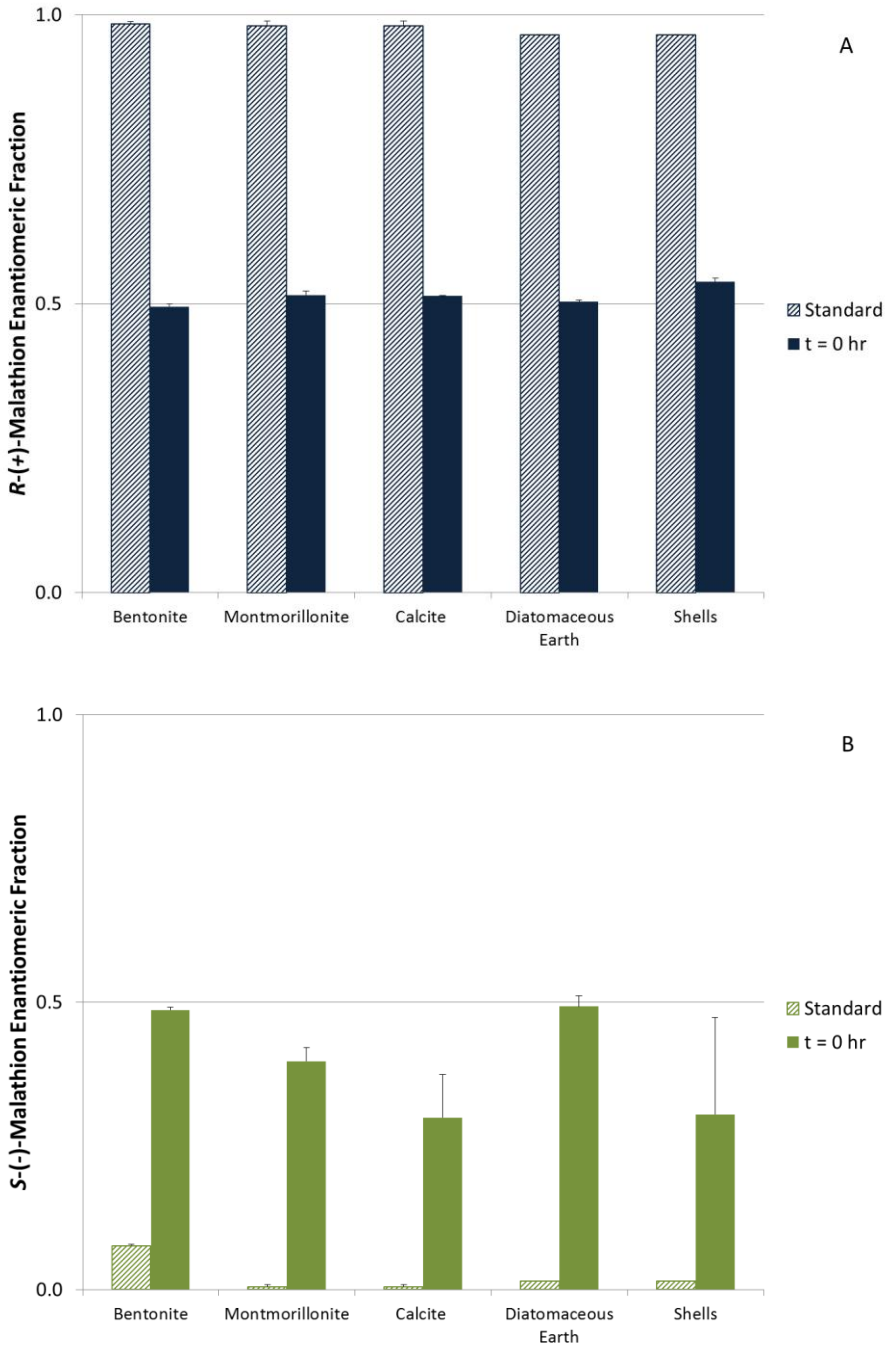


Figure B.6: Enantiomerization of **A**) *R*-(*+*)-malathion and **B**) *S*-(-)-malathion after immediate contact with sorbent. Error bars represent standard deviations of triplicates.

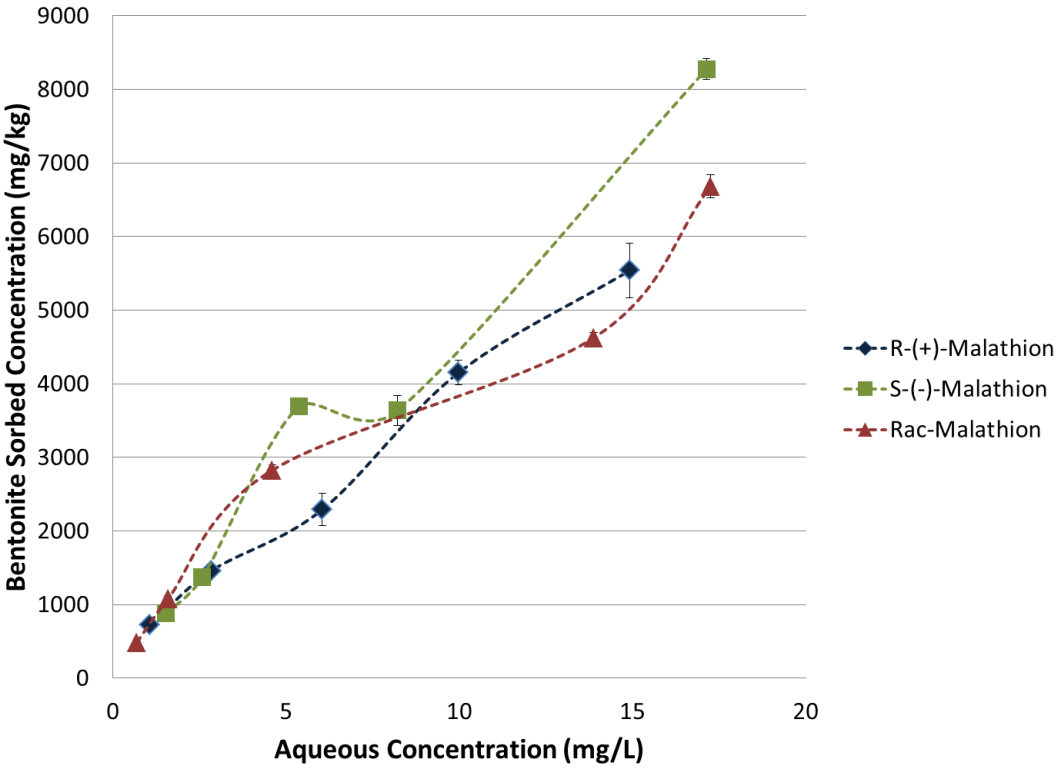


Figure B.7: Bentonite sorption isotherms of *rac*-, *R*-(*+*)-, and *S*-(-)-malathion. Dotted lines represent isotherm shapes and do not represent model fits. Error bars represent standard deviations of triplicates.

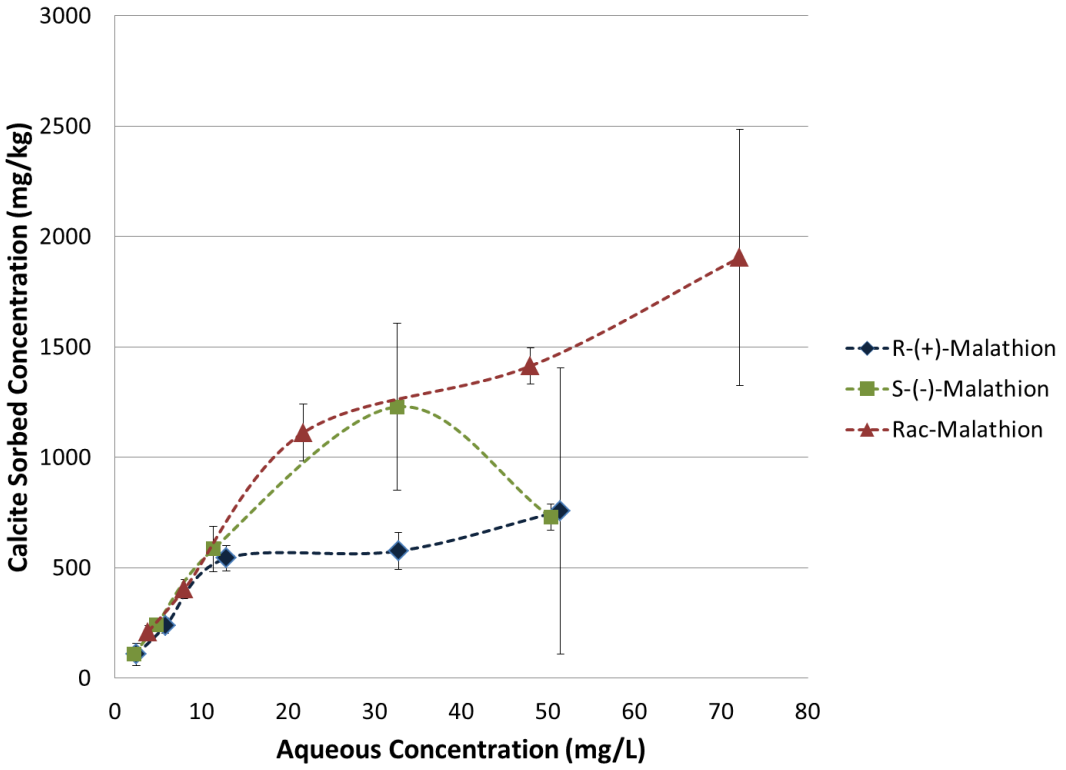


Figure B.8: Calcite sorption isotherms of *rac*-, *R*-(+)-, and *S*-(-)-malathion. Dotted lines represent isotherm shapes and do not represent model fits. Error bars represent standard deviations of triplicates.

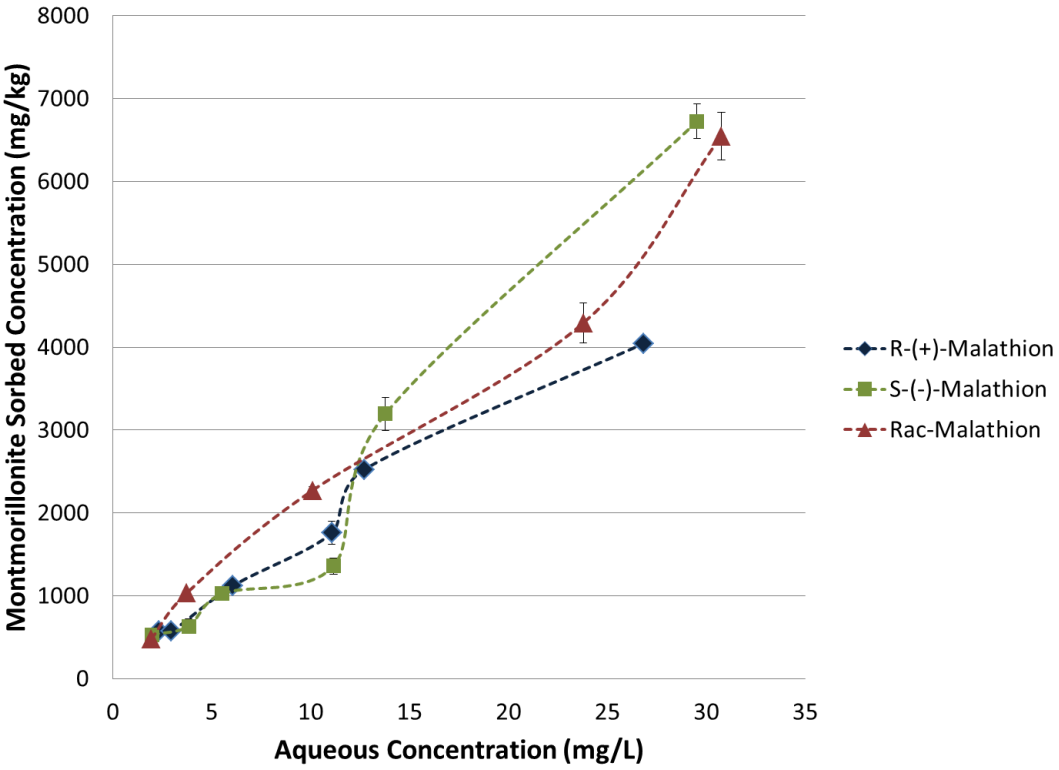


Figure B.9: Montmorillonite sorption isotherms of *rac*-, *R*-(*+*)-, and *S*-(-)-malathion. Dotted lines represent isotherm shapes and do not represent model fits. Error bars represent standard deviations of triplicates.

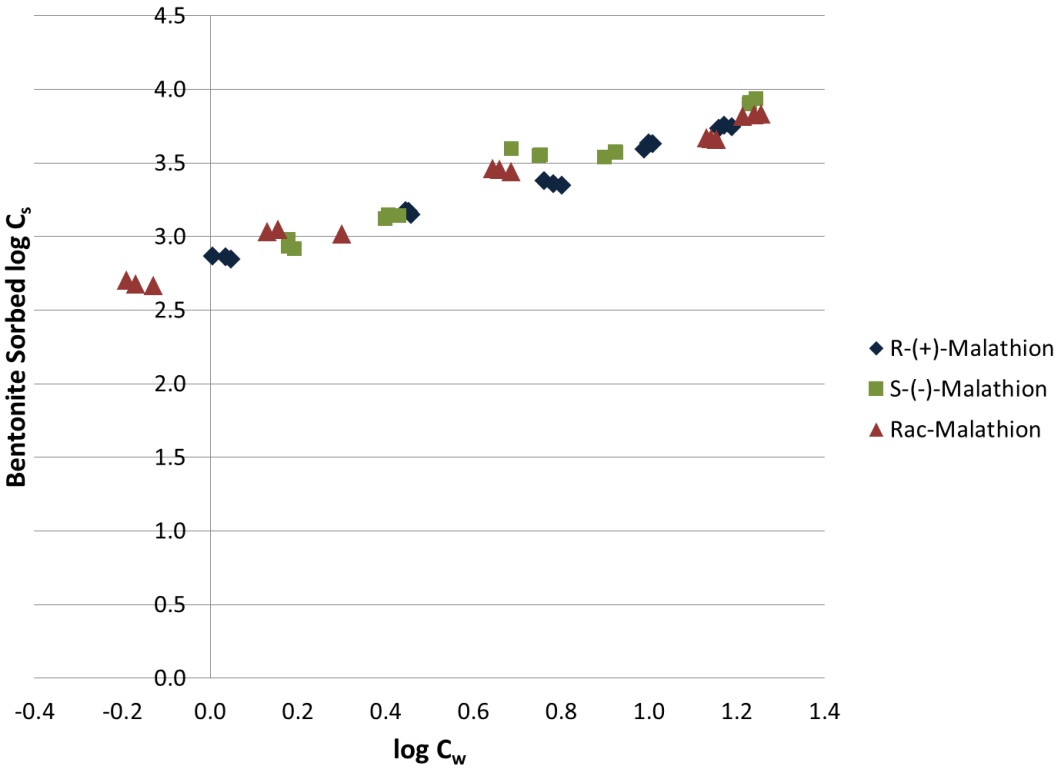


Figure B.10: Bentonite Freundlich sorption isotherms of *rac*-, *R*-(+)-, and *S*-(-)-malathion. C_s = malathion sorbed concentration (mg/kg); C_w = malathion aqueous concentration (mg/L) after two hours equilibration.

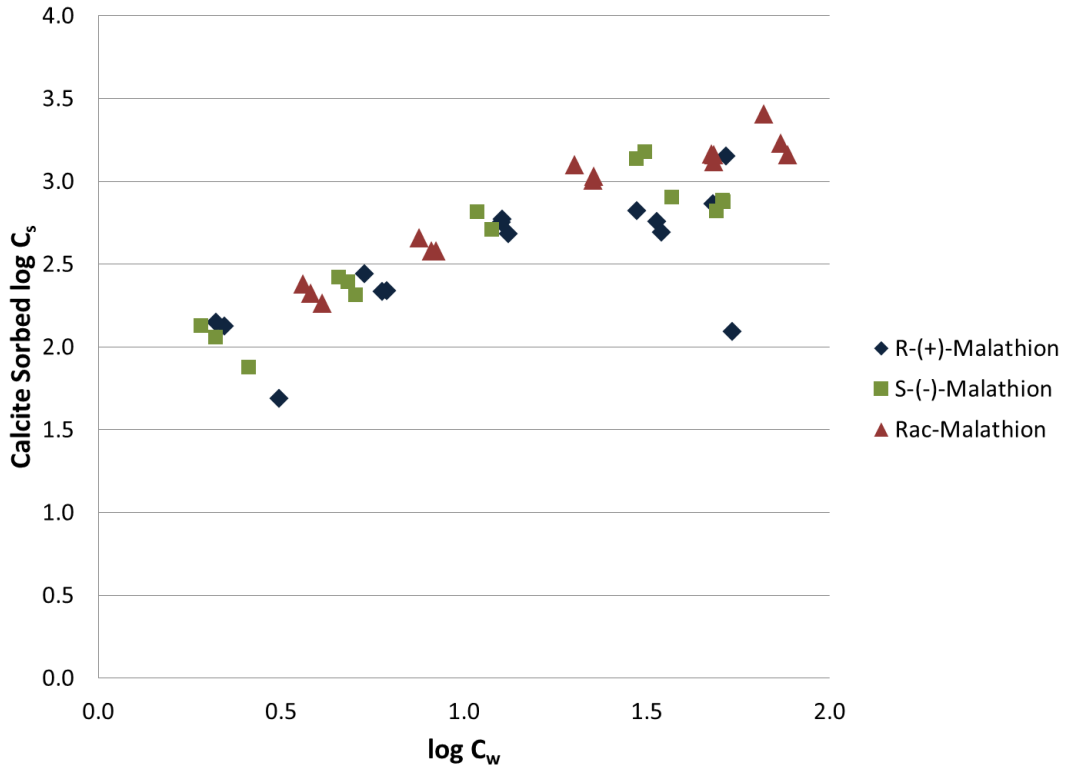


Figure B.11: Calcite Freundlich sorption isotherms of *rac*-, *R*-(+)-, and *S*-(-)-malathion. C_s = malathion sorbed concentration (mg/kg); C_w = malathion aqueous concentration (mg/L) after two hours equilibration.

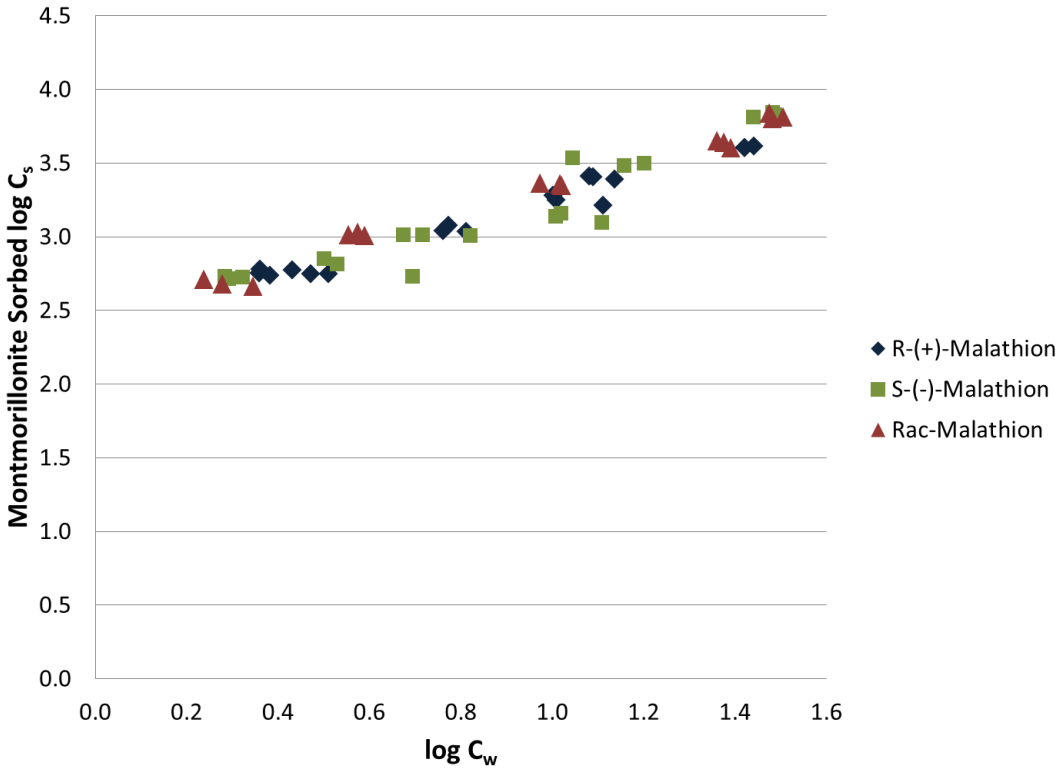


Figure B.12: Montmorillonite Freundlich sorption isotherms of *rac*-, *R*-(*+*)-, and *S*-(-)-malathion. C_s = malathion sorbed concentration (mg/kg); C_w = malathion aqueous concentration (mg/L) after two hours equilibration.

Table B.1: Statistical analysis of malathion Freundlich isotherm parameters.

Sorbent	Comparison	*n* p-values	K_F p-values
Bentonite	*Rac-R*	0.871	0.265
	Rac-S	0.017*	0.203
	R-S	0.019*	0.552
Calcite	*Rac-R*	0.194	0.830
	Rac-S	0.399	0.968
	R-S	0.331	0.968
Montmorillonite	*Rac-R*	0.456	0.739
	Rac-S	0.527	0.738
	R-S	0.266	0.440

A "*" indicates that the difference is statistically significant at $\alpha = 0.05$.

Appendix C

Supplementary Material for Chapter 4

Table C.1: Sorption of *rac*-metalaxyl to 50 mg bentonite.

Time (hr)	*Rac*-Metalaxyl Sorbed (mg/kg)	Enantiomeric Fraction Aqueous Phase
0	2250	0.51
2	2552	0.51
4	2268	0.50
8	2415	0.51
24	2418	0.50
32	2341	0.50
48	2419	0.50

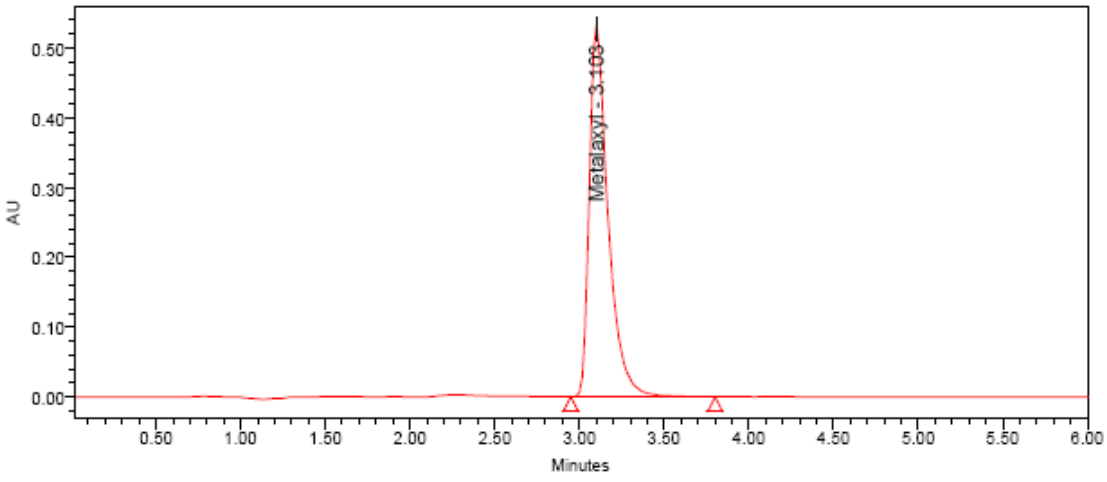


Figure C.1: Achiral HPLC chromatogram of metalaxyl.

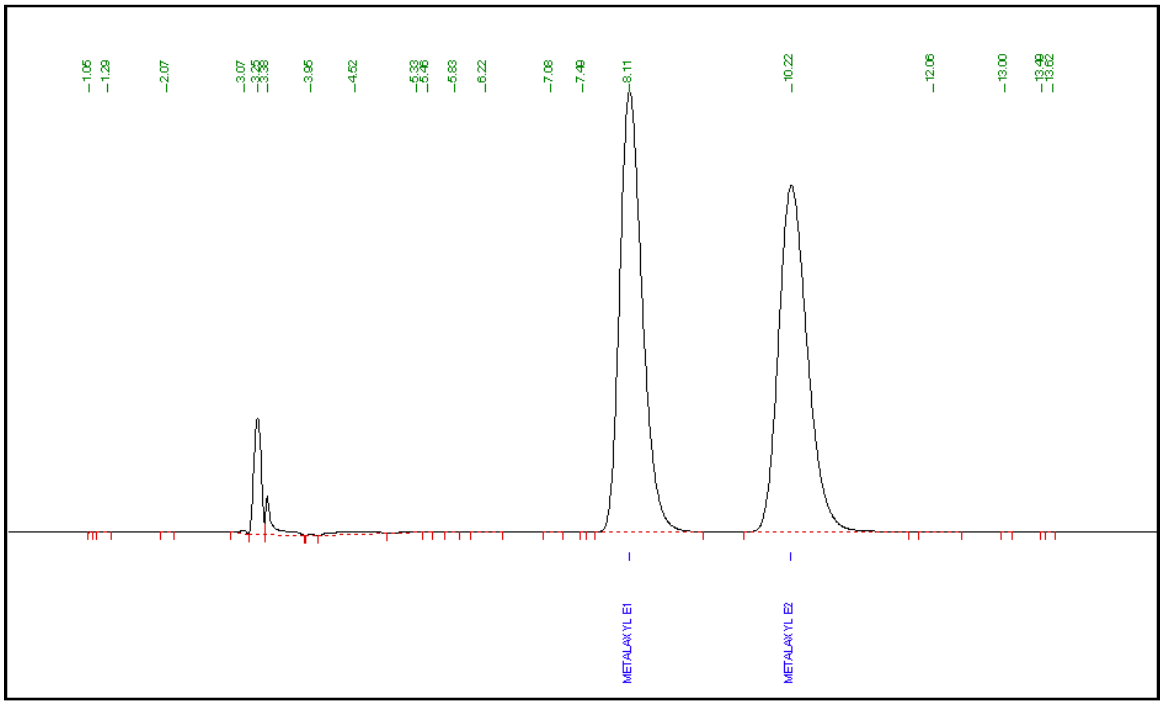


Figure C.2: Chiral HPLC chromatogram of *rac*-metalaxyl.

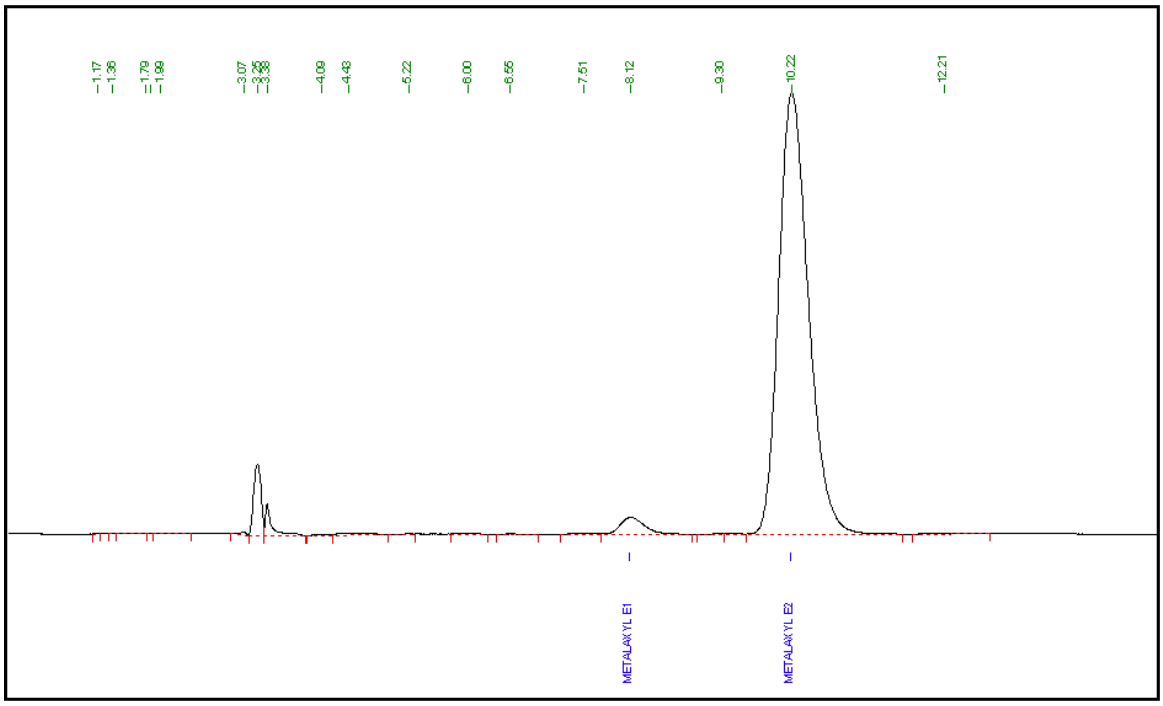


Figure C.3: Chiral HPLC Chromatogram of metalaxyl-M.

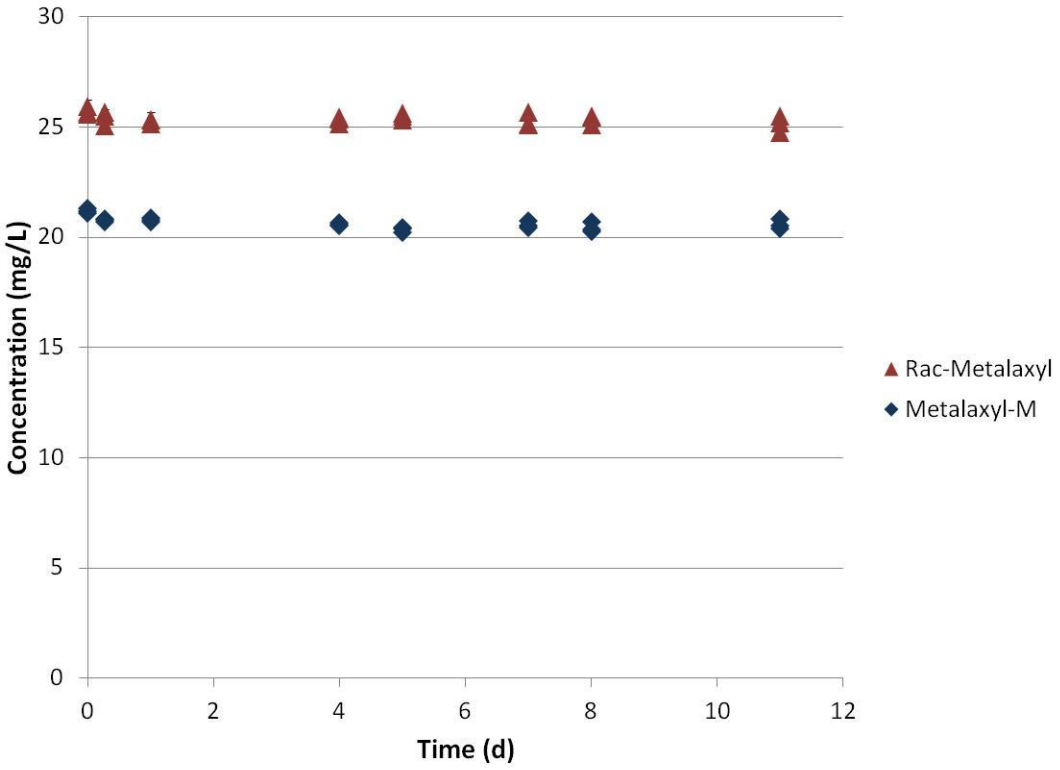


Figure C.4: Stability of *rac*-metalaxyl and metalaxyl-M in water.

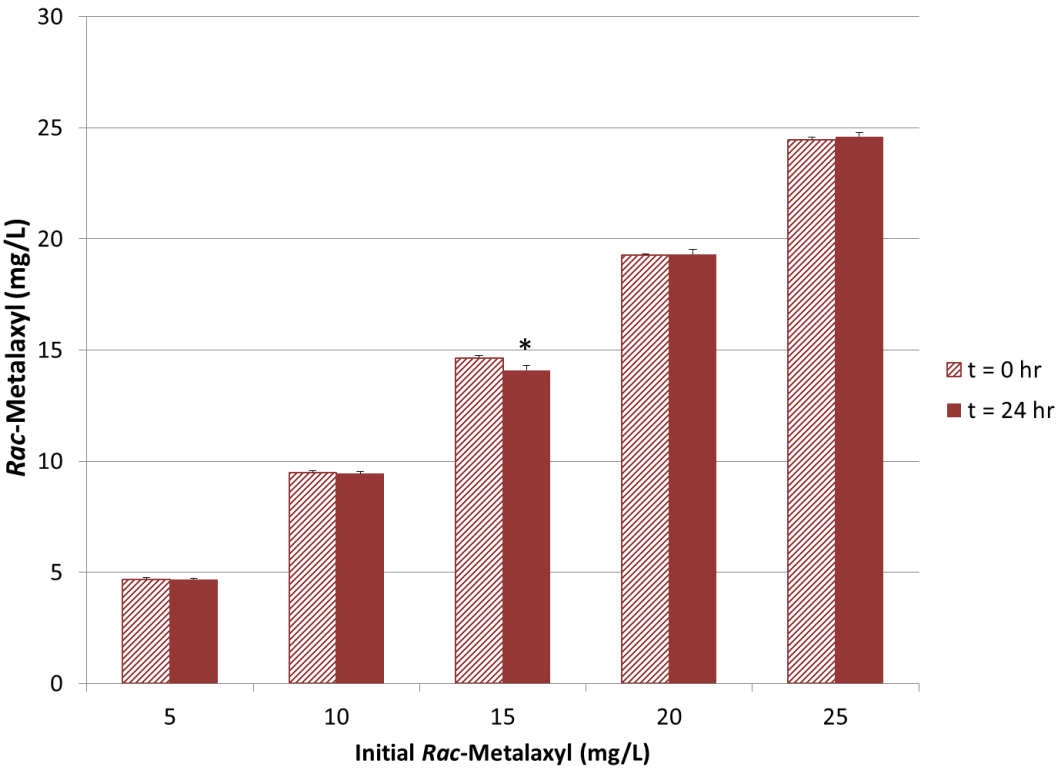


Figure C.5: *Rac*-metalaxyl sorption controls in water. Error bars represent standard deviations of triplicates. A "*" indicates that the difference is statistically significant ($p < 0.05$).

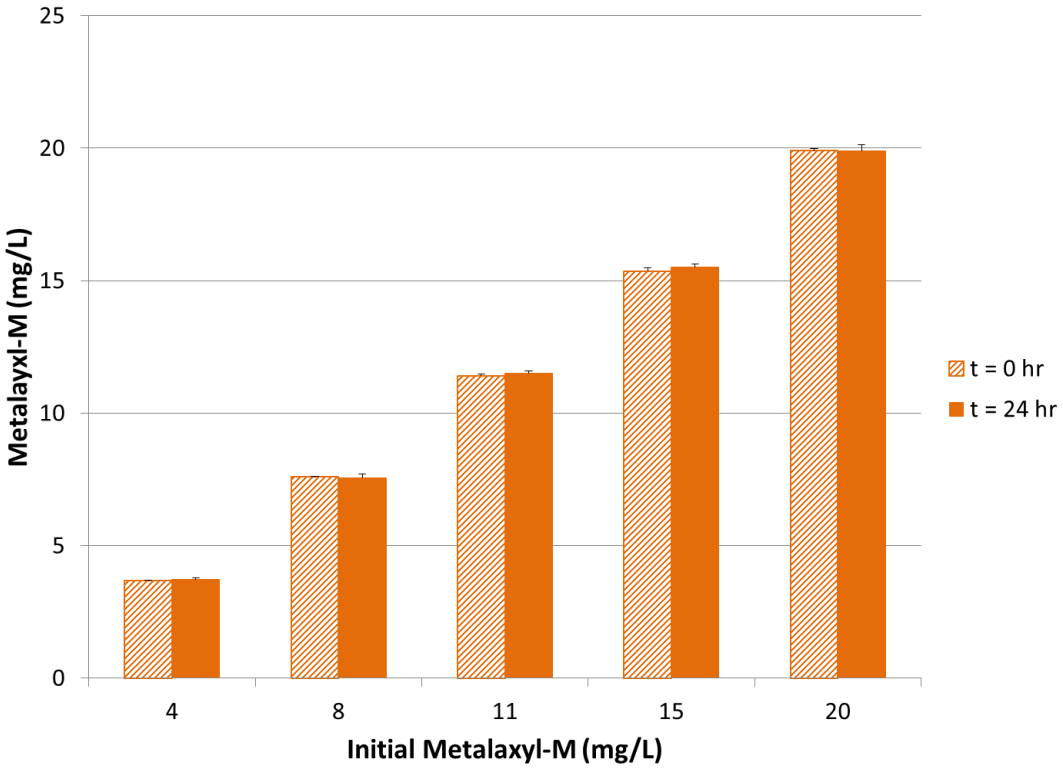


Figure C.6 Metalaxyl-M sorption controls in water. Error bars represent standard deviations of triplicates.

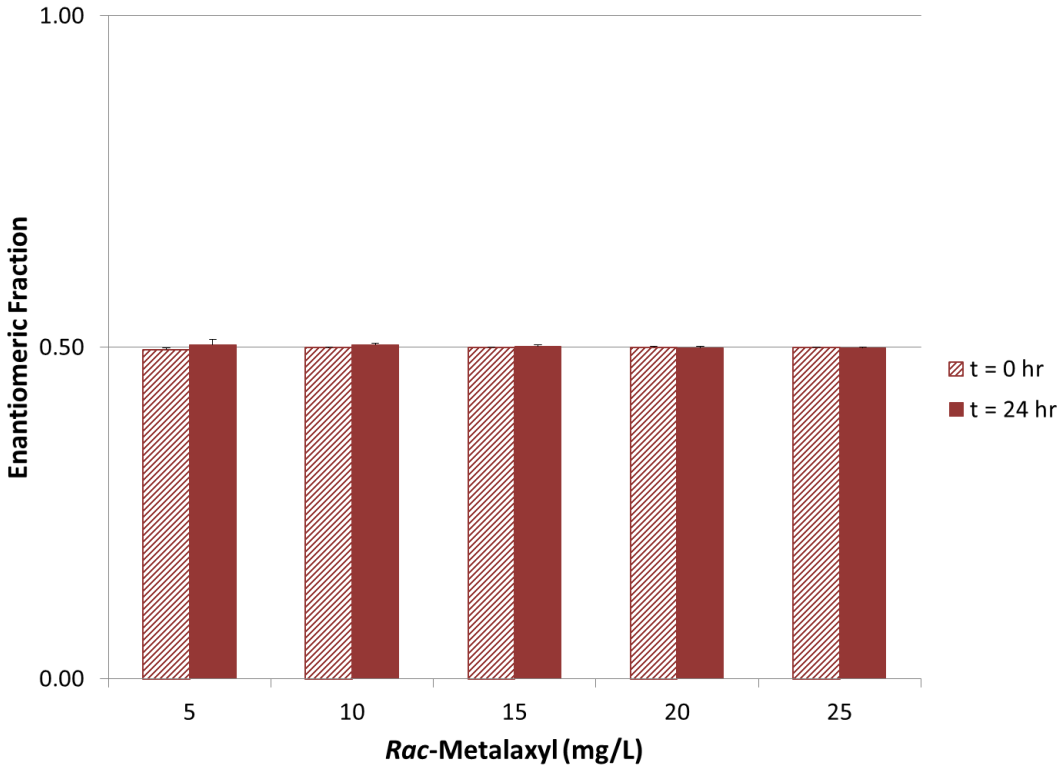


Figure C.7: Enantiomeric fractions of *rac*-metalaxyl sorption controls in water. Error bars represent standard deviations of triplicates.

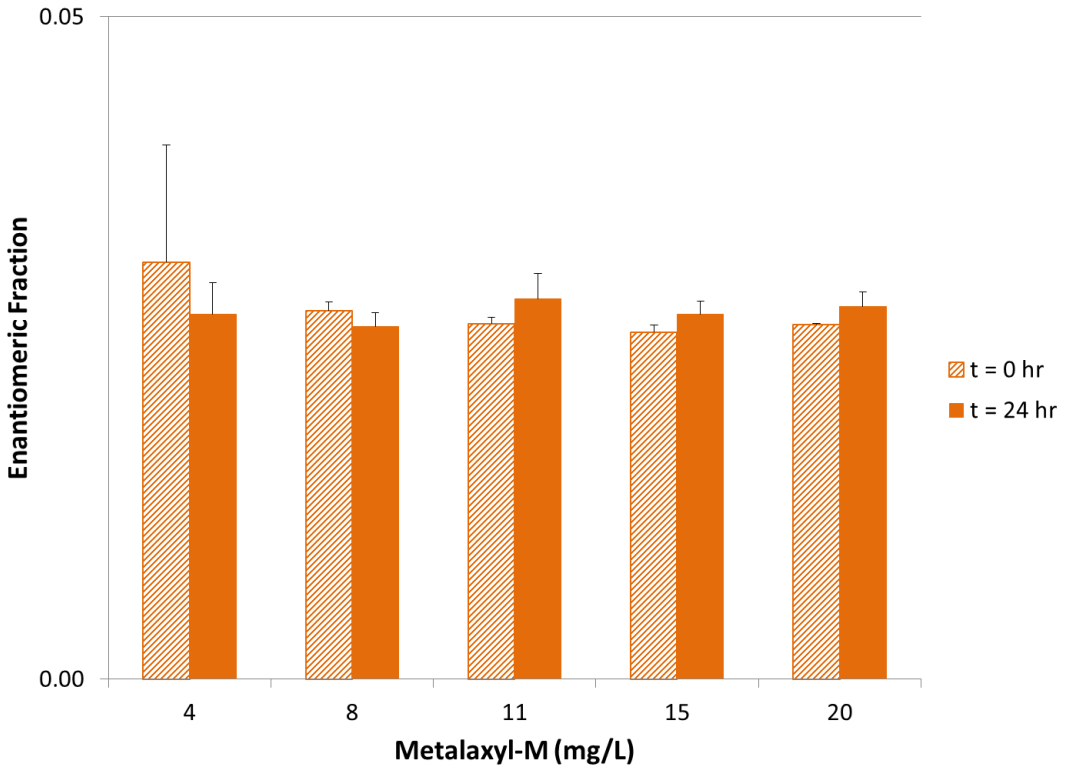


Figure C.8: Enantiomeric fraction of metalaxyl-M sorption controls in water. Error bars represent standard deviations of triplicates.

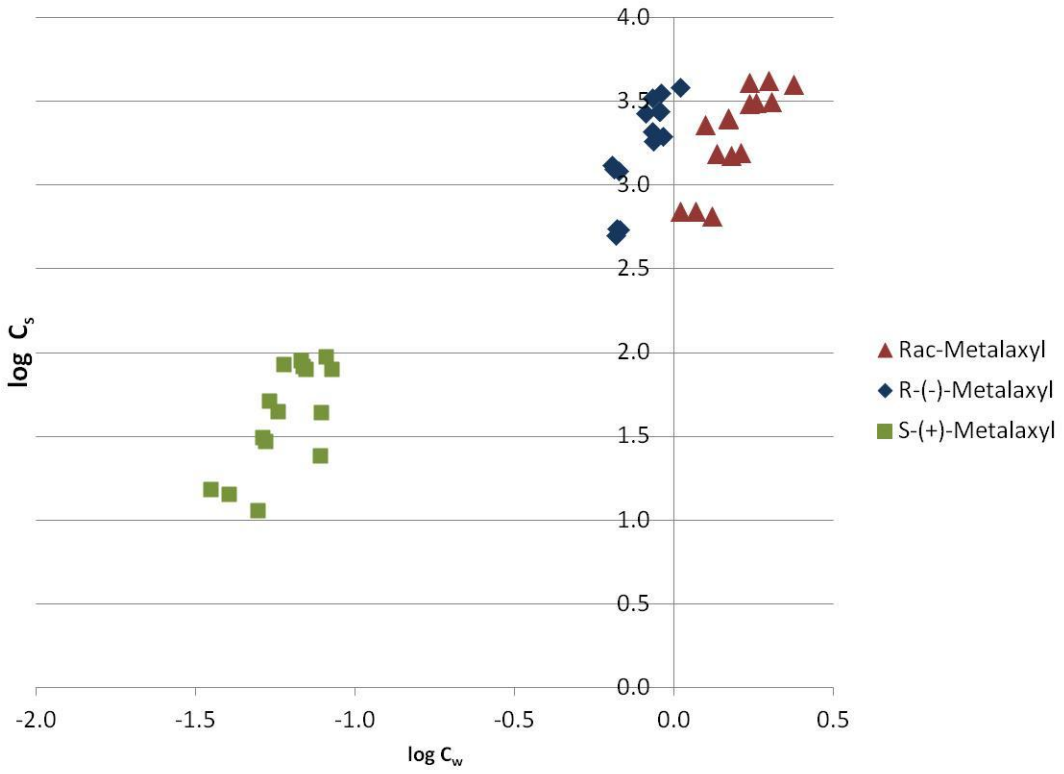


Figure C.9: Bentonite Freundlich sorption isotherms of *rac*-, *R*-(-)-, and *S*-(+)-metalaxyl. C_s = metalaxyl sorbed concentration (mg/kg); C_w = metalaxyl aqueous concentration (mg/L) after 24 hours equilibration.

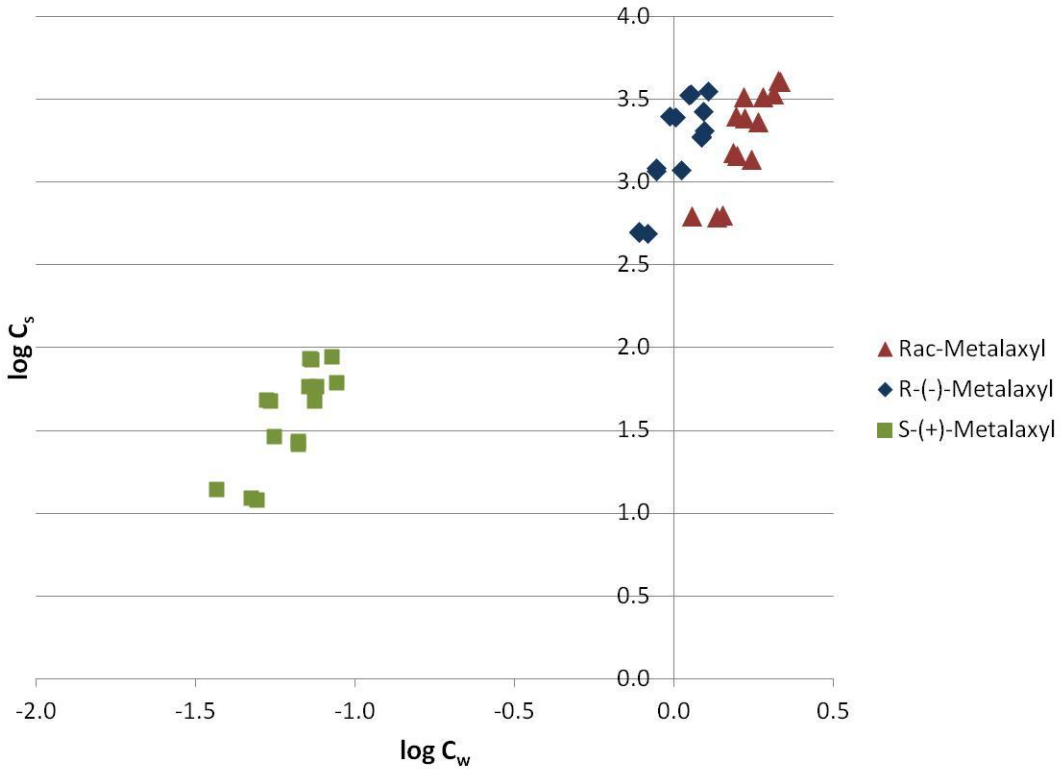


Figure C.10: Montmorillonite Freundlich sorption isotherms of *rac*-, *R*-(-)-, and *S*-(+)-metalaxyl. C_s = metalaxyl sorbed concentration (mg/kg); C_w = metalaxyl aqueous concentration (mg/L) after 24 hours equilibration.

Table C.2: Statistical analysis of metalaxyl Freundlich isotherm parameters.

Sorbent	Comparison	*n* p-values	K_F p-values
Bentonite	*Rac-R*	0.077	0.000006*
	Rac-S	0.204	0.00002*
	R-S	0.204	0.364
Montmorillonite	*Rac-R*	0.870	0.00007*
	Rac-S	0.232	0.00006*
	R-S	0.193	0.032*

A "*" indicates that the difference is statistically significant at $\alpha = 0.05$.

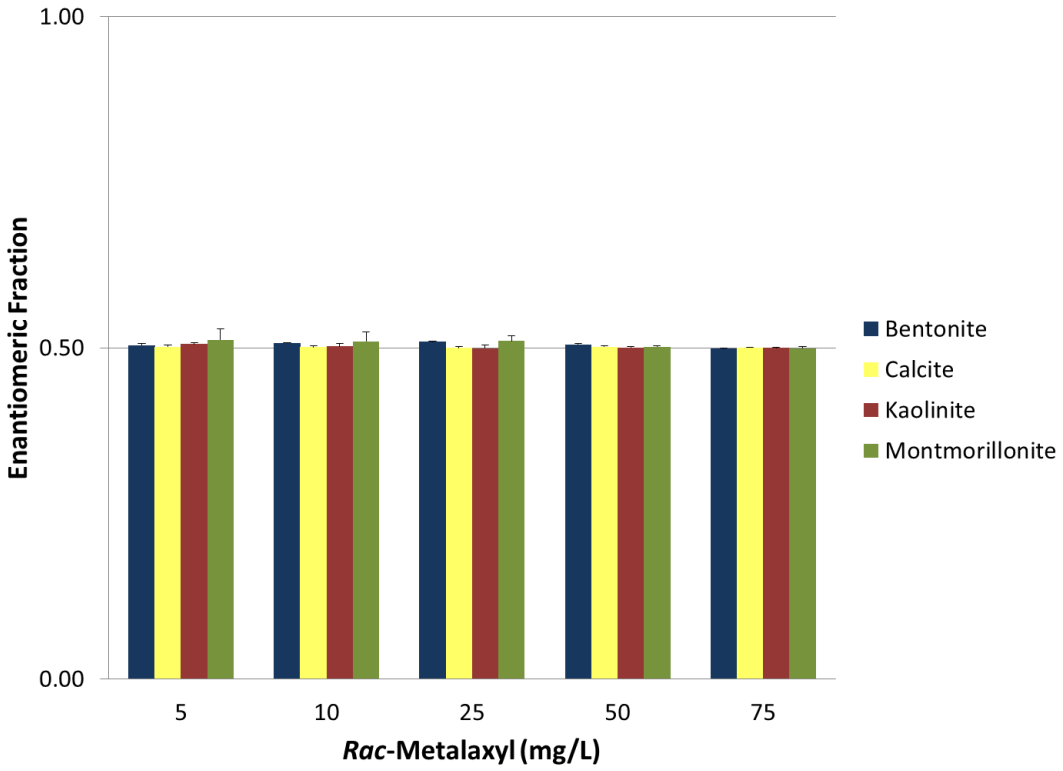


Figure C.11: Enantiomeric fractions of *rac*-metalaxyl after sorption. Error bars represent standard deviations of triplicates.